BIOCHEMICAL AND MICROBIOLOGICAL ASSESSMENTS OF DRIED ALASKAN PINK SALMON, RED SALMON AND PACIFIC COD HEADS

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Abstract

Fish heads are generally considered as unsuitable byproducts for human consumption in the United States. The initial objective was to compare the moisture content and water activity levels on dried pink salmon (*Oncorhynchus gorbuscha*) and dried red salmon (*O. nerka*) using different temperature and time integration. The secondary objective was to compare shelf life characteristics, rancidity and mold growth, between dried pink dried salmon and dried Pacific cod (*Gadus macrocephalus*) heads stored for up to 180 days at the ambient temperature (21°C) for East African seafood markets. The third objective was to assess the antioxidant effects for frozen and dried pink salmon heads stored for up to 60 days. In a preliminary experiment, dried red salmon heads were found unsuitable due to the water activity levels above 0.6. The critical moisture contents were detected around 10% for pink salmon heads and were around 15% for Pacific cod heads to reduce water activity levels below 0.6 in these products. The applicable drying temperatures of 50°C lasting over 50 hours for pink salmon heads and 50°C for over 24 hours followed by 30°C for over 24 hours for Pacific cod heads were found optimal. Dried Pacific cod heads showed shelf stability as a potential dried seafood product. Frozen pink salmon heads had 60 days shelf life, while heads with antioxidant glazing retarded oxidation levels ($p < 0.05$). The antioxidant treatment in dried pink salmon heads kept oxidation levels lower than the acceptable limit up to 60 days. This study provided essential information to improve the utilization of these Alaskan seafood byproducts.

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Chapter 1. General Introduction

1.1 Rationale

Marine resources are in world-class proportions in the oceans off of Alaska. Alaska is the world renowned place for both its resource diversity and pristine environment. Every year, commercial fisheries have provided two million metric tons of finfish and shellfish or more. Alaska's fisheries are sustainably managed through cooperative agreements between agencies: international, federal and state (Grabacki, 2008).

Processing harvested fish into human food is a primary goal for Alaska's seafood processing industry. Edible byproducts of seafood processing include head, viscera, skeletal frames (such as backbones and ribs) and skin. Processing byproducts contain the highest quality in Alaska due to the natural resources management. The amount of waste accumulated changes depending on species and product types. Such waste is enforced to handle according to regulations from the Alaska Department of Environmental Conservation (ADEC) in cooperation with the US Environmental Protection Agency (EPA). Discarding byproducts in local waters reduces the volume of fish products as well as any potential profit they might bring. As competition increases, the extraction of value from all harvested resources will increasingly become a corporate issue. Besides economic loss, discarding processing waste from landed fish can become an environmental problem. In Europe and North America, edible food processing waste averages around 105 kg per year per capita, while in Africa and South Asia, less than 10% of this level is discarded (FAO, 2011).

In many cultures, consumption of fish heads is considered as a treat. Fish heads account for about 20% of round fish weight (Crapo *et al.*, 1993), depending upon species. A fish head can be a quality indicator of a whole fish through smell and color evaluation of gills or eyes (Sveinsdottir *et al.*, 2001), as well as being diagnostic for species identification to reduce mislabeling (Miller and Mariani, 2010). Like any other animal heads (Wein, 1995), fish heads are often used to make soups in many parts of the world including Asia and Africa. In salmonids, sequestered lipids are preferentially stored in the head and under the skin. However, in gadoids, lipids are preferentially stored in the liver (Smiley *et al.*, 2010), while gadoid heads are relatively lean. In Alaska, salmon are canned, headed & gutted and frozen or processed into fillets that are sold either fresh or frozen (ADFG, 2010). Heads, tails, fins and viscera are removed during the processing of salmon. Salmon heads are potentially an excellent source of fish oils for human consumption.

Currently, Alaskan fish heads are either ground and discarded or subjected to further processing to make fish and bone meals and fish oils (Folador *et al.*, 2006). Fish heads are complex containing among other things, the heart, gills, the brain and eyes. In recent years, some salmon processors have begun to render their salmon heads to produce commercial salmon oil for human consumption. This oil is being sold to cosmetics and to nutraceutical companies. Tongue, cheeks and the braincase may have additional value as niche foods. Salmon braincases were reported to have high levels of commercially extractable chondroitin sulfate to be potentially exploited for pharmaceutical purposes (Stine *et al.*, 2010).

Drying is an ancient method of fish preservation. When the product is dried, handling and storage can become relatively inexpensive (McMinn and Magee, 1999). Icelandic fish processors dry their Atlantic cod (*Gadus morhua*) heads for export to East African markets (Arason, 2001). This study primarily focuses on biochemical and microbiological changes in dried pink salmon (*Oncorhynchus gorbuscha*), red salmon (*O. nerka*) heads and compares them with those from dried Pacific cod (*Gadus macrocephalus*) heads. Secondarily, biochemical and microbiological changes in frozen and dried pink salmon heads with/without antioxidant solution were observed. The ultimate goal is to promote the effective utilization of Alaskan fish processing byproducts.

1.2 Literature Review

1.2.1 Overview

The United Nations Population Division predicts that the world population, which is currently over 7 billion (USCB, 2011), will reach approximately 9 billion by 2050 (UN, 2004). Worldwide, undernourishment has risen for the past decade and hunger has increased between 1995-1997 and 2004-2006 in all regions except the Caribbean and Latin America (FAO, 2009a). The United Nations-Food and Agriculture Organization describes food security as physical, social and economic accessibility to sufficient, safe and nutritious food. Global availability of food has become a key issue while the economic, environmental and social sustainability of food have been aggravated by challenges including: energy, distribution, disease and global climate change.

According to the latest United Nations-Food and Agriculture Organization report, worldwide, the production of fish was 143 million metric tons in 2006 (FAO, 2009b). Up to 110 million metric tons of this production was allocated for human consumption, while the remaining 33 million metric tons was used for animal foods including fish meal and fish oil. The total supply of fish has been growing at a rate of 3.6% per year since 1961 and worldwide, annual fish consumption has increased from 9 kg to 16 kg per capita over the interval 1960-1997.

Fish and fishery products are one of the most traded international foods and these products are expected to remain widely traded around the world. Around 37% of worldwide fish production is destined for international trade, while the remaining 63% is headed for domestic markets. Despite fluctuations in supply and demand, seafood products remain essential food resources and represent not only cultural traditions, but also an important revenue stream around the world (FAO, 2002).

Alaska supports one of the most productive commercial fisheries in the world. Alaska's seafood industry ranks third in economic importance behind the North Slope oil and gas industry and federal government (ADOLWD, 2010). Annually, Alaskan harvesters receive over $1 billion for their catch. The value of processed Alaskan seafood sold at first wholesale is estimated to generate around $3 billion annually (ADFG, 2011). The total economic impact of Alaska's seafood industry was estimated at $4.6 billion in 2003. Alaska has commercial harvests of five Pacific salmon species: red or sockeye salmon, pink or humpback salmon, dog or chum salmon (*O. keta),* king or chinook salmon (*O. tshawytscha*) and silver or coho salmon (*O. kisutch*).

Pink salmon, the most abundant salmon harvested in Alaska, are found along the Pacific rim from northeast Asia to the west coast of North America (ADFG, 2011). Pink salmon is the smallest of the Pacific salmon species and commands the lowest price. A total of 162.5 million pink salmons were caught in Alaska in the 2009 commercial season (ADFG, 2011). The ex-vessel value in that year was $140 million. Pink salmon's length ranges between 0.4 m and 0.6 m with an average weight of 1.4 kg to 2.5 kg. In 2009, the value to harvesters for pink salmon was $0.6 per kg from Kodiak salmon processors (ADFG, 2011).

The proximate composition of fish includes lipids, protein, moisture and ash with the proportion of each depending largely on species and season (Huss, 1995). Salmon species generally stop eating once they enter freshwater for spawning. They rely on stored lipids for their energetic needs during this time. Salmon species are anadromous meaning that these marine fish return to freshwater to spawn in their natal streams. The transformation from ocean predator to freshwater spawner is driven by hormone activity and constitutes a series of profound physiological changes to the organism (Reid *et al.*, 1993). Muscle quality can be greatly impacted by these changes. Late run pink salmon have less desirable textures and flavors compared with salmon harvested early in the season. Soft flesh and pale color in late run pink salmon decreases their value (Huynh and Mackey, 1990). A consequence of the cessation of feeding on entering freshwater is ultimately the decrease in lipid content, and the concomitant increase in water and protein (Reid *et al.*, 1993). Distinctive skin watermarking can also occur in late run pink salmon, again due to

the hormonal changes associated with spawning and entry into freshwater (Kitahara, 1983).

In Alaska, groundfish fisheries include pollock, Pacific cod, sablefish, Atka mackerel, as well as numerous rockfish and flatfish species. Pacific cod is widely distributed over the Gulf of Alaska, Bering Sea and the Aleutian Islands. Harvest of this species accounted for 230,000 metric tons or nearly 15% of the 2009 Alaskan groundfish landings. Pacific cod fillets were worth over $327 million in 2008 (NOAA, 2008). The average price paid Alaskan harvesters for Pacific cod was $1.1 per kg in 2008. The maximum length of Pacific cod ranges between 1.3 m and 2 m. Livers from Pacific cod have a long history of being consumed fresh or canned and utilized as the primary source of fish oil (Bechtel and Oliveira, 2006). Frozen Pacific cod heads have been exported to Asian markets from Alaska. During processing of Pacific cod, heads and collar are removed together mechanically, while in salmon processing the mechanical removal of the heads without a collar leaves significantly less meat on the head. The quality of both fresh and processed seafood products have been improved due to advances in processing technology, packaging and logistics.

1.2.2 Byproducts

The global seafood industry focuses primarily on the value received for their seafood production and any potential value from processing byproducts is generally ignored. However, should a potentially profitable product be identified, its processing economically evaluated and its marketing understood, these companies would probably investigate a new potential revenue stream. Today, increased aquatic protein consumption

could alleviate the malnutrition in many countries (Gelman *et al.*, 2003). Some cultures around the world prefer to shop for the whole fish to ascertain the quality of the fish as well as to unambiguously identify the fish species. Head on fish are generally thought to be important for an accurate assessment of quality. In other cultures, the consumption of the fish head is considered a delicacy and is awarded to the most senior members of the family. Biochemical components identified in fish heads besides the proximate composition include: chondroitin, glucosamine, bioactive lipids and peptides (Bimbo, 2009). Niche products, made from these components in the seafood processing waste stream could provide increased revenue to processors. Seafood waste products are generally rendered into animal feed ingredients in the form of fish meal and fish oil. Worldwide production of fish meal is estimated at approximately 6.5 million metric tons per year (FAO, 2009b) and the majority of this production is mainly used by the aquaculture industry. According to Globefish, the fish meal price index was $1,600 per ton in December 2009 and in the aquaculture industry, soy protein meal and fish meal are competitive in price. Total world production of edible fats and oils is estimated around 100 million metric tons per year (FAO, 2009b). Around 21% of this amount comes from soy beans. Marine edible oils constitute only 2% of the total oil production. The fish oil price index was reported $800 per ton in June 2009. This index fluctuates, largely as a function of the price of petroleum and currently, the fish oil price index was above $1,000 per ton in 2011 (Globefish, 2011).

1.2.3 Drying

Dehydration or drying is a physical method for removing water from foods including seafood products (Platt, 2009). Drying, including sun-drying has been practiced at least since 2000 B.C. (Mehas and Rodgers, 2006). The main goal of drying is to convert perishable foods into shelf stable products without using refrigeration. In other words, the goal is storage at ambient temperature by adjusting the water activity and moisture levels of the product. Recently, interest in drying has been renewed (McMinn and Magee, 1999) due to the advances in the technology. Benefits of drying include easier handling, reduced weight and volume, reduced packaging, relatively low storage and transportation costs and energy savings through the reduction of freezing and refrigeration. Numerous methods can be used to remove moisture from different types of products. Convective drying is still the most preferred technique that is used to preserve seafood byproducts. During convective drying, two processes occur: first, energy is transferred from the surrounding environment to the product, and secondly moisture in the product is transferred to the surrounding air.

Water is the most dominant and important component in food systems. In water, two hydrogen atoms share electrons with one oxygen atom in covalent bonds (Mehas and Rodgers, 2006). Water is a highly polar molecule and the hydrogen bonds in water are unusually strong.

During drying of fish, there is a balance between the heat transferred to the fish and the heat providing vaporization of water and overcoming bonding forces. As fish dries, the moisture content in its center will start to drop. Moisture diffuses from the interior of the

product to the surface where it evaporates as dry air pass over the surface. The rate of drying can change due to changes in air temperature, humidity and air velocity (Lewis, 1991). Drying will continue until equilibrium with the outside air is attained. Drying can take from minutes to days, depending on the type of fish, the geometry of the product being dried and the drying conditions. Rapid drying may have undesirable effects on the texture of fish causing a hard semi-impermeable pellicle to form at the surface. When drying involves heated air and the internal temperature exceeds 55°C, fish flesh may begin to cook with subsequent thermal denaturation of the muscle proteins (Burt, 1988). The amount and the state of the water molecules in the product are important (McMinn and Magee, 1999). Free water is relatively easy to remove but bound water, defined as sorbent or solute-associated water, presents kinetic and thermodynamic properties unlike those of pure water can be quite hard to remove (Doe, 1998).

Water activity (a_w) is a measure of the amount of water available in a food system (Doe, 1998). It should not be confused with moisture content, which is a measure of the total amount of water in food. Moisture sorption is the relationship between the water activity and moisture content (Fortes and Okos, 1980). In food systems, bound water is both physically and chemically attached to the protein substrate as well as any salts or sugars present and, therefore, bound water is not available for microbial growth. Measurement of water activity is primarily used to assess potential control over microbial growth in food allowing the maintenance of the safe preservation of foods.

In drying, the dehydration coefficient (DC) can be determined based as the ratio (Eqn. 1) between the amount of water removed during drying and the initial concentration of water (Meda and Ratti, 2005).

$$DC\ (\%) = \frac{W_o - W_{fd}}{W_o - W_d} \times 100 \qquad \text{Eqn. 1}$$

In the equation 1; W_0, W_{fd} and W_d represent the initial sample weight, sample weight after drying and solid weight, respectively. In addition, the drying constant can be calculated based on Eqn. 2 (Lewis, 1991).

$$\frac{\Delta w}{\Delta t} = k\ x\ (T_a - T_d) \qquad \text{Eqn. 2}$$

In equation 2; Δw, Δt, k, T_a and T_d are difference in weight after drying, difference in drying time, drying constant, actual temperature and dew point, respectively.

Traditionally, fish were sun-dried due to its ease. Contamination of the product by flies or birds can be a problem during sun-drying. Alaskan Natives for generations have dried salmon using smoke and wind.

1.2.4 Lipid Oxidation

The oxidative deterioration of lipids may cause economical, safety and quality concerns in various food products. Although many other chemical reactions can risk the safety and quality of processed foods, lipid oxidation is considered as one of the major chemical problems in food processing (Fennema and Tannenbaum, 1996). Lipid oxidation can significantly reduce food quality characteristics such as flavor, color, texture and nutritive value by generating chemical compounds (Nawar, 1996). Therefore, lipid oxidation is a

critical indicator of shelf life for foods. Addition of antioxidants has been the most preferred way to control this problem and phytochemicals have been used rather than synthetic antioxidants due to concerns over possible negative side effects to the body including cardiovascular system (Frankel, 1999; Pokorny, 2007). However, there has to be more research to prove the adverse effects of the synthetic antioxidants. In one study, natural antioxidants had a similar effect on thermal oxidation compared to synthetic antioxidants extracted from tuna oil (Medina *et al.*, 1999). Dietary antioxidants were inversely associated with stroke among cardiovascular disease free women and hemorrhagic stroke among women with cardiovascular disease (Rautiainen *et al.,* 2011). The antioxidants can retard the lipid oxidation (Flick *et al.*, 1992); especially combination of several antioxidants may create a synergistic effect on reducing the lipid oxidation of food products (Wada and Fang, 1992), although the oxidation cannot be thoroughly stopped. Consequently, the mechanism of lipid oxidation must be entirely understood to control oxidation in food products (Abdalla and Roozen, 1999; Naz *et al.*, 2005).

Rancidity is a quality and safety indicator for foodstuffs except for fried foods, dried cereal and cheeses where certain amounts of rancid compounds are important in their flavor profile (Nawar, 1996). When the unsaturated fatty acids and phospholipids become rancid, small volatile aromatic compounds including aldehydes and ketones which cause off-odors and flavors will form in the reaction. Rancidity gives a pale yellow color to fish and off-flavors (rancid).

Lipid oxidation acceleration depends on several factors (Botham and Mayes, 2009): light, temperature, enzymes (i.e., lipoxygenase), metals, metalloproteins and microorganisms.

The generation of free-radicals that is called autoxidation involves initiation, propagation and termination steps (Frankel, 1985; Khayat and Schwall, 1983). Oxidation was reported to damage to viable cells in vivo causing cancer, inflammatory diseases, atherosclerosis and aging (Benzie, 1996).

Due to the high concentrations of polyunsaturated fatty acids (PUFA), fatty fish products are susceptible to lipid oxidation (Ackman, 1990; Sargent *et al.*, 2002). Thereby, fatty fish preservation has been always a challenge limiting its shelf life due to lipids that are variable depending on species age, spawning period, fish diet and muscle types. The fat content is generally correlated to the moisture content of the fish for identification of fatty or lean fish (Ackman, 1990). Muscles structures of fish also plays crucial role for their shelf life. White muscled fish provide their energy via a glycogen hydrolysis called glycolysis, whereas dark muscled fish rely on an oxidative phosphorylation called β-oxidation of lipids for producing energy (Hultin and Kelleher, 2000) containing more fish oil than white muscle. Thus, white muscled fish are considered as lean fish. Dark muscled fish contain more pro-oxidative myoglobins which can ease lipid oxidation and aggravate rancidity. When fish are oxidized, they become unsuitable for marketing due to their low quality and safety for human consumption. To test oxidation of fish and fishery products, peroxide value (PV), p-anisidine value (PAV), thiobarbituric acid reactive substances (TBARS) and free fatty acid (FFA) levels are used to determine the quality and safety of these products.

Enzymes in fish can be responsible to initiate the oxidation process (Frankel, 1985). The potential role of lipoxygenase is crucial in catalyzing the oxidation of fish muscle lipids.

The occurrence of a very active 12-lipoxygenase in the gill tissue of trout was previously identified (Hsieh *et al.*, 1988). A pro-oxidant in the skin, a heme protein, was isolated from sardine (Mohri *et al.*, 1990). Nevertheless, in the lipid oxidation mechanism, oxygen has the greatest responsibility for triggering oxidation in the food system (McClements and Decker, 2000). Thus, exclusion of oxygen from food products is an important task in the food processing. This can be accomplished by vacuum packaging or modified and controlled atmospheric storage or by glazing with an antioxidant during frozen storage. Heating accelerates lipid oxidation in muscle tissue of fish (Shahidi and Botta, 1994) and cooking of fish muscle can prevent enzymatic lipid oxidation. Freezing is used to reduce the rate of lipid oxidation via immobilization of catalysts and lagging post-mortem changes. However, frozen-thawed fish muscle may oxidize more rapidly than non-frozen tissues upon temperature abuses. Salting fish may also increase the lipid oxidation rate in fish muscle due to sodium ion (Shahidi and Botta, 1994).

The overall goal of this project was to determine the suitability of dried fish heads destined to East African markets for human consumption. Alaskan natural fishery resources, byproducts, drying technology and lipid oxidation were essential topics to identify the background information for this project. Alaskan pink salmon, red salmon and Pacific cod head parts were investigated for the project.

1.3 References

Abdalla A.E. and Roozen J.P., 1999. Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. Food Chem. 64: 323-329.

Ackman R.G., 1990. Seafood lipids and fatty acids. Food Rev. Int. 6: 617-646.

ADFG, 2010. Run forecasts and harvest projections for 2010 Alaska salmon fisheries and review of the 2009 season. Eggers D.M., Plotnick M.D. and Carroll A.M. (Eds.). pp. 1-93. Alaska Department of Fish & Game. Available at: www.state.ak.us/adfg/adfhome.htm.

ADFG, 2011. Salmon fisheries in Alaska. Harvest and species overview. Alaska Department of Fish and Game. Available at: www.adfg.alaska.gov.

ADOLWD, 2010. Research and analysis section. The U.S Department of Labor. Bureau of labor statistics. Quarterly census of employment and wages. Alaska Department of Labor and Workforce Development. Available at: www. laborstats.alaska.gov.

Arason S., 2001. Drying of fish and utilization of geothermal energy- The Icelandic experience. Keynote lectures. 1st Nordic drying conference. Trondheim. 27th-29th June 2001.

Bechtel P.J. and Oliveira A.C.M., 2006. Chemical characterization of livers from fish species harvested in Alaska. J. Food Sci. 71(6): 480-486.

Benzie I.F.F., 1996. Lipid peroxidation: A review of causes, consequences, measurement and dietary influences. Int. J. Food Sci. 47(3): 233-261.

Bimbo P.A., 2009. Alaska seafood byproducts: Potential products, markets and competing products. Report for Alaska Fisheries Development Foundation. Anchorage, AK. pp. 277.

Botham M.K. and Mayes P.A., 2009. Biologic oxidation. Chapter 12. In: Harper's illustrated biochemistry. 28th edition. Murray R.K., Bender D.A., Botham M.K., Kennelly P.J., Rodwell V.W. and Weil P.A. (Eds.). pp. 98-101.

Burt J.R., 1988. Fish smoking and drying: The effect of smoking and drying on the nutritional properties of fish. Burt J.R. (Ed.). Elsevier Publishers. UK. pp. 166.

Crapo C.A., Paust B. and Babbitt J.K., 1993. Recovery and yields from Pacific fish and shellfish. Alaska Sea Grant College Program. Marine Advisory Bulletin 37. University of Alaska, Fairbanks. pp. 10.

Doe P.E., 1998. Fish drying and smoking: Production and quality. Technomic Publishing Inc. UK. pp. 250.

FAO, 2002. The state of the world fisheries and aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy. pp. 20.

FAO, 2009a. Undernourishment around the world. The state of food insecurity in the world. pp. 8-28. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO, 2009b. The state of the world fisheries and aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy. pp. 30.

FAO, 2011. Global food losses and food waste. Gustavsson J., Cederberg C., Sonesson U., Van Otterdijk R. and Meybeck A. (Eds.). Food and Agriculture Organization of the United Nations, Rome, Italy. pp. 29.

Fennema O.R. and Tannenbaum S.R., 1996. Introduction to food chemistry. Chapter 1. pp. 1-15. In: Food chemistry. 3rd edition. Fennema O.R. (Ed.). Marcel Dekker. New York. pp. 1069.

Flick G.J., Hong G.P. and Knobl G.M., 1992. Lipid oxidation of seafood during storage. Lipid Ox. Food. 11: 183-207.

Folador J.F., Karr L.L.K., Persons C.M., Bauer L.L., Utterback P.L., Schasteen C.S., Bechtel P.J. and Fahey J.G.C., 2006. Fish meals, fish components and fish hydrolysates as potential ingredients in pet foods. J. Animal Sci. 84: 2752-2765.

Fortes M. and Okos M.R., 1980. Drying theories: Their bases and limitations as applied to food and grains. In: Advances in drying. Mujumdar A.S. (Ed.). Hemisphere Publishing. New York. pp. 119-154.

Frankel E.N., 1985. Chemistry of free radical and singlet oxidation of lipids. Prog. Lipid Res. 23: 197-221.

Frankel E.N., 1999. Natural phenolic antioxidants and their impact on health. In: Antioxidant food supplements in human health. Academic Press. London. pp. 385-392.

Gelman A., Cogan U., Mokady S., Drabkin V. and Glatman L., 2003. Obtaining human food from whole under-utilized fish. In: Advances in seafood byproducts. 2002 conference proceedings. Bechtel P.J. (Ed.). Alaska Sea Grant College Program. University of Alaska Fairbanks. Fairbanks, AK. pp. 403-420.

Globefish, 2011. Market reports of fish oil and fish meal. Available at: www.globefish.org.

Grabacki T.S., 2008. Sustainable management of Alaska's fisheries. Available at: www.alaskaseafood.org. pp. 27.

Hsieh R.J., German J.B. and Kinsella J.E., 1988. Lipoxygenase in fish tissue: Some properties of the 12-lipoxygenase from trout gill. J. Agr. Food Chem. 36: 680-685.

Hultin H.O. and Kelleher S.D., 2000. Surimi processing from dark muscle fish. In: Surimi and surimi seafood. Park J.W. (Ed.). Marcel Dekker, New York. pp. 59-77.

Huss H.H., 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper. No. 438. Rome, Italy. Food and Agriculture Organization of the United Nations. pp. 195. Available at: www.fao.org.

Huynh M.D. and Mackey J., 1990. A quality study of late run chum salmon. In: Seafood Science and Technology. Bligh E.G. (Ed.). Fishing News Book. pp. 163-175.

Khayat A. and Schwall D., 1983. Lipid oxidation in seafood. Food Technol. 37(7): 130-140.

Kitahara T., 1983. Behavior of carotenoids in the chum salmon during anadromous migration. Biochem. Physiol. 76(B1): 783-791.

Lewis W.K., 1991. The rate of drying of solid materials. Ind. Eng. Chem. Symposium on drying. 3(5): 42-43.

McClements D.J. and Decker E.A., 2000. Lipid oxidation in oil-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food system. J. Food Sci. 65(8): 1270-1282.

McMinn W.A.M. and Magee T.R.A., 1999. Principles, methods and applications of the convective drying of foodstuffs. Trans Ind. Chem. Eng. 77(C): 175-193.

Meda L. and Ratti C., 2005. Rehydration of freeze-dried strawberries at varying temperatures. J. Food Proc. Eng. 28(3): 233-246.

Medina I., Gracia M.T.S., German J.B. and Frankel E.N., 1999. Comparison of natural polyphenol antioxidants from extra virgin olive oil with synthetic antioxidants in tuna lipids during thermal oxidation. J. Agr. Food Chem. 47: 4873-4879.

Mehas K.Y. and Rodgers S.L., 2006. Food science: The biochemistry of food and nutrition. 5th Edition. McGraw Hill Publishing. Peoria, IL. pp. 495.

Miller D.D. and Mariani S., 2010. Smoke, mirrors and mislabeled cod: Poor transparency in the European seafood industry. Front. Ecol. Env. 8(10): 517-521.

Mohri S., Cho S.Y., Endo Y. and Fujimoto K., 1990. Lipoxygenase activity in sardine skin. Agr. Bio. Chem. 54: 1889-1891.

Nawar W.W., 1996. Lipids. In: Food Chemistry 3rd Edition. Fennema O.R. (Ed.). New York, Marcel Dekker. pp. 225-319.

Naz S., Siddiqi R., Sheikh H. and Sayeed S.A., 2005. Deterioration of olive, corn and soybean oils due to air, light, heat and deep-frying. Food Res. Int. 38(2): 127-134.

NOAA, 2008. Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands area: Economic status of the groundfish fisheries of Alaska. Available at: www.afsc.noaa.gov.

Platt C.G., 2009. Food Science and Technology. Wiley-Blackwell Publishing. UK. pp. 508.

Pokorny J., 2007. Are natural antioxidants better and safer than synthetic antioxidants? J. Lipid Sci. Tech. 109: 629-642.

Rautiainen S., Larsson S., Virtamo J. and Wolk A., 2011. Total antioxidant capacity of diet and risk of stroke: A population based prospective cohort of women. J. Am. Heart Assoc. Stroke. 43: 1-7.

Reid R.A., Durance T.D., Walker D.C. and Reid P.E., 1993. Structural and chemical changes in the muscle of chum salmon during spawning migration. Food Res. Int. 26: 1-9.

Sargent J.R., Tocher D.R. and Bell J.G., 2002. The lipids. In: Fish nutrition. 3rd Edition. Halver J.E. and Hardy R.W. (Eds.). Elsevier. UK. pp. 181-257.

Shahidi F. and Botta J.R., 1994. Seafoods: Chemistry, processing, technology and quality. Chapman and Hall. London, UK. pp. 342.

Smiley S., Demir N., Oliveira A.C.M. and Bechtel P.J., 2010. Characterization of dried heads from five Pacific salmon species, dried at different temperatures. In: A sustainable future: Fish processing byproducts. Smiley S. and Bechtel P.J. (Eds.). Alaska Sea Grant. University of Alaska Fairbanks. pp. 55-66.

Stine J.J., Wu T.H., Oliveira A.C.M., Smiley S. and Bechtel P.J., 2010. Extraction and determination of chondroitin sulfate from fish processing byproducts. In: A sustainable future: Fish processing byproducts. Bechtel P.J. and Smiley S. (Eds.). Alaska Sea Grant. University of Alaska Fairbanks. pp. 41-53.

Sveinsdottir K., Martinsdottir E., Hyldig G., Jorgensen B. and Kristbergsson K., 2001. Quality index method (QIM) and quantities descriptive analysis (QDA) in shelf life study of farmed Atlantic salmon (*Salmo salar*). Eurofish. Denmark. pp. 48.

UN, 2004. World population to 2030 in New York. United Nation. Available at: www.un.org.

USCB, 2011. 2007 population estimates program. The United States of Census Bureau. Available at: www.census.gov.

Wada S. and Fang X., 1992. The synergistic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. J. Food Proc. Pres. 16: 263-274.

Wein E.E., 1995. Evaluating food use by Canadian aboriginal people. Can. J. Physio. Pharma. 73: 759-764.

Chapter 2. Drying Pink and Red Salmon Heads

2.1 Introduction

Fish processing byproducts in Alaska total around 1.5 million metric tons (mt) annually (Bechtel, 2003). A number of these processing byproducts were previously investigated in Alaska (Bechtel, *et al.*, 2009; Bechtel and Oliveira, 2006; Oliveira *et al.*, 2009; Smiley *et al.*, 2010). In Alaska, between 50,000 and 80,000 mt of pink and red salmon heads are annually produced resulting in 20,000 mt of unrefined salmon oil (Sathivel *et al.*, 2005). Byproducts of farmed Atlantic salmon (*Salmo salar*) are processed into salmon oil for human consumption in Norway (Skara *et al.*, 2004). The goal of this study was to investigate dried salmon heads as a convenient soup ingredient for East Africa.

As a food item, dried food products have been considered microbiologically safe due to low water activity levels. Controlling the water activity is critical to prevent mold presence in dried products (Sautour *et al.,* 2002). According to Abbas *et al.,* (2009), when water activity of a food item is reduced to 0.6, both bacterial and mold growth can be prevented and thus shelf life can be extended.

This preliminary study was conducted to determine the most suitable time-temperature integration to dry pink and red salmon heads and compare them with one another. The water activity and moisture content of dried pink and red salmon heads were measured under different time and temperature regiments. Proximate composition of wet and dried pink and red salmon heads was measured. Drying temperatures tested were 40°C, 50°C,

60°C and sampling times of 0, 24, 36, 48, 60 and 72 hours in six replicates were done. The major concern was mold presence in dried salmon heads.

2.2 Materials and Methods

2.2.1 Sample Preparation

Pink and red salmon heads were collected from a local processor in Kodiak, AK. In all cases, six individual heads (six replicates from the same batch) were used for analyses. After collection, the heads were immediately frozen and stored at -40°C. Average weights of frozen red and pink heads were ~236 g and ~118 g, respectively. A batch of 36 red and pink salmon heads was thawed at 4°C. A custom air-dryer (Enviro-Pak Model CHN-150 E, Clackamas, OR) was used to dry the head samples. The following drying procedure was applied for both pink and red salmon heads: gills were manually removed, the heads were bisected, the bisected heads were placed on drying racks and temperature, relative humidity and dew point were recorded for up to 72 hours with 100% blower speed. Six frozen pink and red salmon heads were removed for wet samples analyses and thawed at 4°C. Both wet and dried samples were masticated using a laboratory grinder (Cuisine Art, China). The drying coefficient (DC) and the drying constant (k) of dried pink and red salmon heads were determined by calculation (Eqn.1 and Eqn. 2) as described in Chapter 1. The DC is the ratio between the water content of wet samples and the amount of water removed during drying. The k describes the mechanisms of heat and mass transport phenomena depending on both material and air properties.

2.2.2 Water Activity

The water activity was measured on pulverized samples using a water activity meter (Aqua Lab Model 3TE, Decagon Devices Inc., Pullman, WA).

2.2.3 Proximate Composition

The moisture and ash contents were determined according to standard AOAC methods 952.08 and 938.08, respectively (AOAC, 2005). The protein content (%) was calculated using a LECO Nitrogen Analyzer (TruSpec, St. Joseph, MI). A 6.25 multiplying factor was used to calculate the protein content. Lipids were extracted using Accelerated Solvent Extraction (ASE Model 200, Dionex, Sunnyvale, CA) and also using the acid hydrolysis method no. 922.06 (AOAC, 1922; AOAC, 1926).

2.2.4 Thiobarbituric Acid Reactive Substances (TBARS) Analysis

The lipid rancidity was determined using thiobarbituric acid (TBA) method on wet samples (Lemon, 1975). Malonaldehyde (MA) content of the samples was expressed in units of mg MA/kg tissue.

2.2.5 Free Fatty Acid (FFA) Determination

The free fatty acids (FFA) levels of wet samples were measured according to AOCS (1998) method number Ca 5a-40. The FFA values were reported as % oleic acid.

2.2.6 Microbiological Analysis

Total viable counts (TVC) and mold growth of wet and dried samples were determined. Data were expressed as Log Colony Forming Units per gram (CFU/g).

2.2.7 Statistical Analysis

Statistica version 9.0 (Tulsa, OK) was used for determination of means for all measured parameters. An analysis of variance (ANOVA) with Tukey honesty significant difference (HSD) test was used for evaluating significant differences ($p < 0.05$).

2.3 Results and Discussion

2.3.1 Proximate Analysis of Wet Fish Heads

The proximate composition and water activity of wet pink and red salmon heads are reported in Table 2.1. The lipid content of wet red salmon heads averaged 17% ± 1.5% by acid hydrolysis method, while the ASE method gave an average value of 20% ± 2.7% in wet red salmon heads. The proximate composition results of wet pink and red salmon heads showed that wet red salmon heads were higher in lipids and lower in moisture and protein content when compared to wet pink salmon heads.

2.3.2 Lipid Oxidation of Wet Fish Heads

The TBA values of wet red salmon heads averaged 1.4 ± 0.1 mg MA/kg, while the TBA value of wet pink salmon heads averaged 2.4 ± 0.3 mg MA/kg. The FFA value of wet red salmon heads averaged 4.9% ± 0.8%, while mean FFA value of wet pink salmon heads was 6.9% ± 0.8% after 30 days storage at -40°C.

2.3.3 Microbiological Content of Wet Fish Heads

The TVC for wet red salmon heads averaged 5.3 Log CFU/g, while wet pink salmon heads' had a mean TVC of 5.2 Log CFU/g. Initial microbial loads of both wet pink and red salmon heads showed that they were below 7 Log CFU/g (ICMSF, 1978).

2.3.4 Drying of Fish Heads

The moisture and the water activity were recorded during drying under different temperature regimes for pink and red salmon heads. In Figure 2.1, moisture sorptions (absorption and adsorption considered as a single process) of pink and red salmon heads are shown. When red salmon heads were dried for up to 72 hours, the water activity stayed above 0.6 and averaged at 0.7 ± 0.1. The moisture content of red salmon heads was averaged at 9.0% ± 0.8% after 72 hours drying at 50°C. When pink salmon heads were dried up to 72 hours, the water activity went below 0.6 and the moisture content reduced to 10% or less. The moisture content of pink salmon heads decreased to 5.9% ± 0.9% and the water activity was 0.3 ± 0.1 after 72 hours drying at 50°C. The average moisture, water activity, drying coefficient and drying constants of red and pink salmon heads dried under a 50°C temperature are reported in Tables 2.2 and 2.3.

The proximate composition and water activity of dried pink and red salmon heads are presented in Table 2.4. The average water activity, moisture and lipid content of dried red salmon heads were higher than for dried pink salmon heads. No TVC were measured for dried pink and red salmon heads. However, mold growth was observed in dried pink and red salmon heads after 30 days which were dried at 30°C, 40°C and 50°C for less than 48

hours. When the drying procedure was carried out at temperatures below 50°C, active bacterial growth might occur (Tarr, 1954). Above 50°C, nutritional loss might develop during drying due to the denaturation of proteins (Barham, 2001; Lea *et al.,* 1958; Opstvedt, 1988). The moisture content of 10% or less in pink salmon heads was critical to achieve the reduction of the water activity to below 0.6 that is required to inhibit mold growth. The drying pink salmon heads at 50°C for 50 hours was determined to be the optimal time and temperature integration.

2.4 Conclusion

This study showed differences in the proximate compositions between pink and red salmon heads. The differences in oil and moisture contents are important since they will affect drying time, drying efficiency and mold growth.

Plotting the moisture content versus water activity showed that the slope of the drying graph decreased sharply during the initial 24 hours. Subsequently, the water activity levels changed quite gradually compared to the first 24 hours. Most likely, free water was removed quickly, while the bound water removal took longer (Doe, 1998). In the case of drying red salmon heads at 50°C, it was difficult to reduce the water activity to below 0.6. This study suggests that dried pink salmon heads are more suitable for further experiments involving biochemical and microbiological analyses due to their lower water activity levels when compared to dried red salmon heads. A 50°C drying temperature lasting for 48-60 hours and a moisture content of 10% or less appear to be critical to achieve the reduction in water activity of pink salmon heads to below 0.6.

TBA and FFA results showed that, given the amount of lipids in these heads, the stability against oxidative rancidity might be a concern in both pink and red salmon heads. The microbiological results showed that the wet material could contain an initial microbial load below the generally acceptable limit of 10^7 CFU/g. Milder drying conditions and water activity levels above 0.6 resulted in mold detection in the samples.

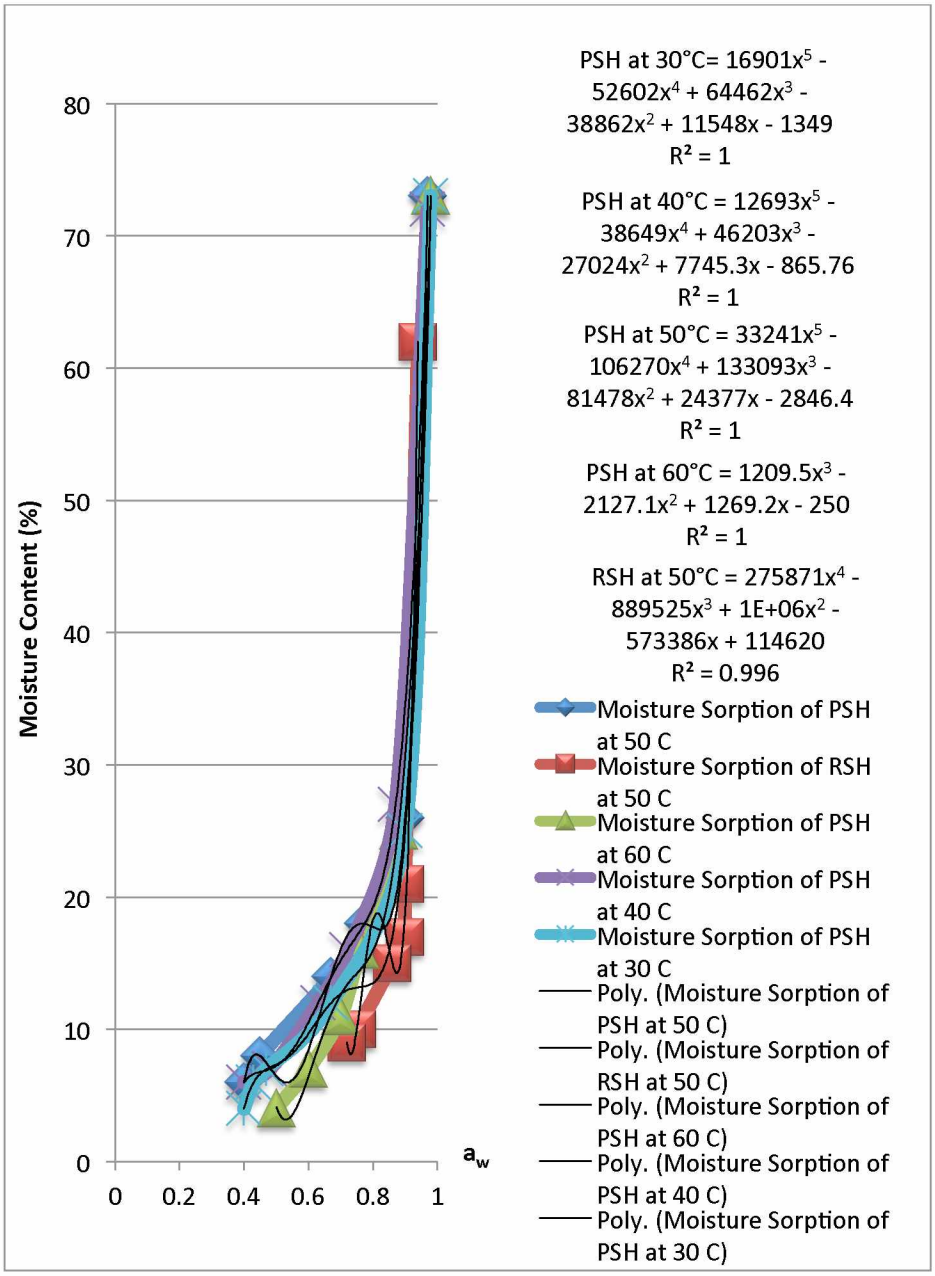

Figure 2.1 Moisture sorptions for pink and red salmon heads

n=6; PSH: Pink Salmon Heads; RSH: Red Salmon Heads; MS: Moisture Sorption.

Table 2.1 Proximate analysis for pink and red salmon heads

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
PSH	1.0^a	0.0	71.7^a	0.9	9.4^a	1.1	13.8^a	1.4	5.1^a	0.2
RSH	1.0^a	0.0	62.2^b	2.3	20.5^b	2.7	11.9^b	1.2	5.4^a	0.3

n=6; PSH: Pink Salmon Heads; RSH: Red Salmon Heads; SD: Standard Deviation;

Different letters within column indicate statistical differences at $p < 0.05$.

Table 2.2 Drying kinetics for red salmon heads at 50°C

Time (hours)	Average a_w	SD	Moisture Content (%)	SD	DC (%)	k
24	0.9	0.0	21	2.6	12	0.001
36	0.9	0.0	17	1.3	30	0.007
48	0.9	0.0	15	0.8	48	0.012
60	0.8	0.1	10	0.6	63	0.016
72	0.7	0.1	9	0.8	76	0.020

a_w: Water Activity; SD: Standard Deviation; DC: Drying Coefficient; k: Drying Constant.

Table 2.3 Drying kinetics for pink salmon heads at 50°C

Time (hours)	Average a_w	SD	Moisture Content (%)	SD	DC (%)	k
24	0.9	0.0	26	2.3	89	0.069
36	0.7	0.0	12	3.3	96	0.009
48	0.5	0.0	8	3.0	98	0.002
60	0.5	0.1	7	1.0	98	0.001
72	0.3	0.1	5	0.9	99	0.001

a_w: Water Activity; SD: Standard Deviation; DC: Drying Coefficient; k: Drying Constant.

Table 2.4 Proximate analysis for dried pink and red salmon heads at 50ºC for 50 hours

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
DPSH	0.5^a	0.0	6.5^a	0.9	44.5^a	1.7	36.6^a	1.7	12.4^a	0.5
DRSH	0.7^b	0.0	15.4^b	1.5	46.6^b	1.0	29.4^b	1.6	8.6^b	0.6

n=6; DPSH: Dried Pink Salmon Heads; DRSH: Dried Red Salmon Heads; SD: Standard Deviation; Different letters within column indicate statistical differences at $p < 0.05$.

2.5 References

Abbas K.A., Saleh A.M., Mohammed A. and Lasekan O., 2009. The relationship between water activity and fish spoilage during cold storage: A review. J. Food Agr. Env. 7(3&4): 86-90.

AOAC, 1922. Official methods of analysis of AOAC International. Association of Official Agricultural Chemists International, Gaithersburg, MD.

AOAC, 1926. Official methods of analysis of AOAC International. Association of Official Agricultural Chemists International, Gaithersburg, MD.

AOAC, 1998. Official Methods of analysis of AOAC International. 1-2. AOAC (Association of Official Agricultural Chemists) International, Gaithersburg, MD.

AOAC, 2005. Official methods of analysis of AOAC International. 18th edition. Association of Official Analytical Chemists International, Arlington, VA.

AOCS, 1998. Official methods and recommended practices of the American Oil Chemists' Society (5th Edition). Champaign, Illinois, Washington, DC.

Barham P., 2001. The science of cooking. Springer-Verlag. UK. pp. 237.

Bechtel P.J., 2003. Advances in seafood by-products: 2002 Conference proceedings. Alaska Sea Grant College Program, University of Alaska Fairbanks. pp. 566.

Bechtel P.J. and Oliveira A.C.M., 2006. Chemical characterization of livers from fish species harvested in Alaska. J. Food Sci. 71(6): 480-486.

Bechtel P.J., Morey A., Oliveira A.C.M., Wu T.H., Plante S. and Bower C.K., 2009. Chemical and nutritional properties of Pacific Ocean Perch (*Sebastes alutus*) whole fish and byproducts. J. Food Pro. Pres. 34: 55-72.

Doe E.P., 1998. Fish drying and smoking production and quality. Doe E.P. (Ed.). Technomic Publishing, PA. pp. 250.

ICMSF, 1978. Microorganisms in foods. Vol. 1. The International Commission on Microbiological Specifications for Foods. Toronto, ON, Canada. University of Toronto Press. pp. 343.

Lea C.H., Parr L.J. and Carpenter K.J., 1958. Chemical and nutritional changes in stored herring meal. Brit. J. Nutr. 12(3): 297-311.

Lemon D.W., 1975. An improved TBA test for rancidity. Environment Canada. Fisheries and Marine Service. New Series Circular No. 51: 52-55. Halifax, Nova Scotia.

Meda L. and Ratti C., 2005. Rehydration of freeze-dried strawberries at varying temperatures. J. Food Proc. Eng. 28: 233-246.

Oliveira A.C.M., Bechtel P.J., Morey A. and Demir N., 2009. Composition of heads and livers of Yelloweye Rockfish (*Sebastes ruberrimus*) harvested in Alaska. J. Aq. Food Product Tech. 18(1): 53-66.

Opstvedt J., 1988. Influence of drying and smoking on protein quality. In: Fish smoking and drying. Burt J.R. (Ed.). Chapter 2. pp. 23-24.

Sathivel S., Smiley S., Prinyawiwatkul W. and Bechtel P.J., 2005. Functional and nutritional properties of red salmon enzymatic hydrolysates. J. Food Sci. 70(6C): 401-406.

Sautour M., Mansur S.C., Divies C., Bensoussan M. and Dantigny P., 2002. Comparison of the effects of temperature and water activity on growth rate of food spoilage molds. J. Ind. Micro. Biotech. 28: 331-315.

Skara T., Sivertsvik M. and Birkeland S., 2004. Production of salmon oil filleting byproducts effects of storage conditions on lipid oxidation and content of ω-3 polyunsaturated fatty acids. J. Food Sci. 69(8E): 417-421.

Smiley S., Bechtel P.J., Desantos F.A. and Brewer M.S., 2010. Effects of inclusion of salmon roe on characteristics of salmon baby food products. J. Food Sci. 75(4): 231-236.

Tarr H.L.A., 1954. Microbiological deterioration of fish post mortem, its detection and control. Microbiol. Mol. Biol. Rev. 18(1): 1-15.

Chapter 3. Storage Stability of Dried Pink Salmon and Pacific Cod Heads

3.1 Introduction

In this study, dried pink salmon heads (DPSH) and Pacific cod heads (DCH) were compared for biochemical and microbiological stability at ambient temperature storage for 180 days. The dried form is used as a fish soup ingredient. Soup is a popular food item with all age groups from different cultures all around the world. Shiau and Chai (1990) studied oyster soup made from oyster processing waste. Mol reported (2005) on salmon soup made from the edible portions of salmon processing waste kept under refrigeration was marketable for international markets. Arason (2001) reported that Icelandic processors are using drying technology on Atlantic cod (*Gadus morhua*) for export to Africa. This market pays 18 Norwegian Krone ~ $3 per kilo for these dried fish heads, whereas the price for fish meal made into animal feed (sold to EU countries) is around 2 Norwegian Krone ~ $0.40 per kilo (Fiskeriforskning, 2004). In Iceland, 5,600 mt of raw Atlantic cod heads can be converted into 1,120 mt of dried heads with the value of 15-20 million Norwegian Krone ~ $2.6-3.4 million. Indigenous people, as well as many Asian cultures include fish parts such as the head, tongue and livers in their diet (Wein, 1995). The findings suggest new opportunities for using fish parts to increase the value of fish landed in Alaska.

3.2 Materials and Methods

3.2.1 Processing

Pink salmon heads (PSH) and Pacific cod heads (CH) were freshly collected from a processing plant in Kodiak, AK. In all cases, six replicates were selected for analyses.

The gills were manually removed, the head split into halves and placed on drying trays. A custom air-dryer (Enviro-Pak Model CHN-150 E, Clackamas, OR) was used to dry the samples. For PSH, the final target weight was measured based on preliminary findings of moisture-water activity correlations as described in Chapter 1. The CH were dried at 20°C for over 50 hours, 50°C for over 24 hours following 30°C for over 24 hours and 70°C for over 12 hours to observe the drying curves and to determine the best time and temperature integration for drying. The PSH were masticated using a laboratory grinder (Cuisine Art, China), while CH were ground using a milling device (Thomas-Wiley, Swedesboro, NJ) equipped with 2 mm grinding plates. The PSH were dried at 50°C for over 50 hours (N=90). The CH (N=50) were dried at 50°C for over 24 hours and subsequently at 30°C for over 24 hours totaled 53 hours. The samples were subjected to biochemical and microbiological analyses at six time points: 0, 14, 30, 60, 90 and 180 days. Dried head samples were placed in double gusset bags and stored in the incubator at ambient temperature (21°C) until analyzed. The lipid oxidation levels and the total microbial load and mold growth were monitored. Two halves of PSH and CH were used as one sample unit in the replications.

3.2.2 Proximate Analysis

The proximate compositions of wet and dried samples were determined. The AOAC methods 952.08 and 938.08 were used to measure the moisture and ash contents (AOAC, 2005). The protein content was carried out using an automated protein analyzer (Elementar Rapid N III, Hanau, Germany). A 6.25 multiplying factor was used to

calculate % protein content. The lipid content was determined using an Accelerated Solvent Extractor (ASE Model 200, Dionex, Sunnyvale, CA).

3.2.3 Water Activity

The water activity levels of both wet and dried samples were determined at room temperature using an Aqualab water activity meter (Model Series 3TE, Decagon Devices Inc., Pullman, WA).

3.2.4 TBARS Analysis

The Thiobarbituric acid reactive substances determination was used to measure secondary oxidation levels in the samples (Lemon, 1975). The malonaldehyde (MA) content of the samples was expressed in units of mg MA/kg tissue. The TBARS values below 6 mg MA/kg of fish tissue are acceptable under food safety and quality guidelines (Freeman and Hearnsberger, 1994). The maximum allowable malonaldehyde content is 8 mg MA/kg of fish tissue (Schormuller, 1968).

3.2.5 Peroxide Value (PV) Determination

The PV was measured to detect primary oxidation products in the samples using the Richards *et al.*, (2007) method. The unit of PV was expressed as mequiv oxygen/kg of sample tissue. Maximum PV limit is 20 mequiv oxygen/kg of fish tissue (Connell, 1995).

3.2.6 FFA Analysis

The concentration of FFA is a measure of lipid autolysis (Doe, 1998). Lipid hydrolysis is responsible for increased % FFA (Fennema, 1996). The FFA content of extracted oils was determined according to AOCS method number Ca 5a-40 (AOCS, 1998). The FFA

values were reported as % oleic acid. Maximum FFA acceptable levels are between 7-8 % for fish oils (Bimbo, 2009).

3.2.7 Fatty Acid Methyl Esters (FAMEs) Analysis

The FAMEs were prepared using a modification of Maxwell and Marmer's method (1983). Gas chromatography (GC) analysis used a GC model 7890 A (Agilent, Santa Clara, CA) fitted with a FAMEWAX^{TM} 30 m x 0.32 mm ID x .25 μm GC column (Restek Bellefonte, CA). A GC ChemStation program (Ver E.02.00.493, Agilent, Santa Clara, CA) was used to analyze data. Helium was used as a carrier gas at an average velocity of 64 cm/sec. Injector and detector temperatures were held at 250°C and 280°C, respectively. A split injection (50:1 split ratio) was used. Oven temperature was adjusted from 50°C to 220°C at a rate of 5°C/minutes and held at 220°C for additional 2 minutes giving a total run of 36 minutes. An autosampler performed the GC injection of standards and samples. The injection volume was 1μL. The FAMEs were identified by comparing retention times to standards. The standards were: Supelco 37, PUFA # 1, PUFA # 3 and Pacific cod liver oil from Supelco (Bellefonte, PA). The data were expressed as percent of total integrated area.

3.2.8 Microbiological Analysis

Five grams of the wet head samples were weighed into a filter bag and filled with sterilized 0.1% peptone water up to fifty grams total weight. The wet head samples were homogenized by using a stomacher (IUL Instruments, Barcelona, Spain) for two minutes. Aerobic plate counts were determined by spread plating technique onto pre-poured Bacto Plate Count Agar medium with 0.5% salt (Difco Laboratories, Detroit, MI) in triplicates.

Plates were incubated at 25°C for 72 hours to evaluate the initial microbial load of wet samples and count bacterial colonies (Messer *et al.*, 1984).

Two grams of dried head samples were weighed into a filter bag and filled with sterilized 0.1% peptone water up to twenty grams of total weight. Total plate count was determined using the same technique as for wet head samples. Sterilized Potato Dextrose Agar medium (Difco Laboratories, Detroit, MI) which was lowered to pH 3.5 with 10% tartaric acid solution was used to enumerate the mold colonies in the dried head samples. These plates were incubated at 35°C for 48 hours to count the bacteria and molds from the dried head samples (Messer *et al.*, 1984). Color determination sticks (EM Science, Darmstadt, Germany) were used to ensure the pH requirements for agar media. The acceptable limit for mold growth was set at zero in our experiments. The maximum aerobic plate count limit for fresh and frozen fish is 7 Log (CFU)/g as employed by International Commission on Microbiological Specifications for Foods (ICMSF, 1978).

3.2.9 Amino Acid Analysis

The amino acid profiles were determined by the AAA Service Laboratory Inc. (Damascus, OR). The amino acids were quantified using a Bechman 6300 Analyzer with post column ninhydrin derivization. Triplicates of freeze-dried (wet samples) and dried pink salmon and Pacific cod heads stored for 180 days at ambient temperature were analyzed. Tryptophan and cysteine were not determined.

3.2.10 Mineral Analysis

The mineral analysis was conducted at the University of Alaska Fairbanks, School of Natural Resources and Agricultural Sciences, Palmer Research Center. The dried head

samples were burned overnight at 550°C. The ash residues were digested in an aqueous solution containing 10% (v/v) hydrochloric and nitric acids. Six replicates of DCH and twelve replicates of DPSH stored for 180 days at ambient temperature were analyzed for Ca, P, Mn, K, Mg, Fe, Cu, Zn, Pb, Hg, As, Cd, Sr and Ni by inductively coupled plasma optical emission spectrometry using a Perkin Elmer Optima 3000 Radial ICP-OES (Waltham, MA).

3.2.11 Statistical Analysis

Statistica Version 9.0 (Tulsa, OK) was used for determination of means for all measured parameters. An ANOVA test with Tukey HSD test was used for evaluating significant differences ($p < 0.05$).

3.3 Results and Discussion

3.3.1 Drying

A 60°C drying temperature was reported as the starting point for losing nutritional quality through cooking of drying fishery products (Burt, 1988). Drying Atlantic cod (*Gadus morhua*) heads at 20°C was reported to take 5 days in Iceland using a commercial dryer and geothermal energy (Arason, 2001). In this experiment, the moisture content of CH averaged 35% after 50 hours of drying at 20°C, which had an average water activity of 0.7. However, these mildly dried CH became covered with visible mold after 3 days of ambient temperature storage. Drying CH at 70°C took around 20 hours until the moisture content averaged 16% and the average water activity was below 0.6. However, drying at 70°C was unacceptable due to cooking the product with the proteins becoming denatured (Burt, 1988). When the drying temperature was increased, the drying curve changed from

linear to exponential and the moisture removal at higher temperatures took less time (Kingsly *et al.*, 2007). Drying curves for CH are presented in Fig 3.1. The moisture content below 20% in CH was critical to achieve a reduction in the water activity to below 0.6, the standard value for inhibition of mold growth (Abbas *et al.*, 2009). A 50°C and subsequent 30°C drying of CH over 50 hours were selected as the best time and temperature integration. Figure 3.2 shows the drying curve for PSH at 50°C. The polynomial equations of drying trials are presented to allow prediction of the time and temperature integration by measuring the net weight at desired drying time. Moisture removal during drying was calculated based on the moisture content of the wet material for both PSH and CH. The 50°C was the critical drying temperature that prevented cooking or denaturing of the product, while allowing the drying to be completed in a feasible amount of time.

3.3.2 Proximate Analysis

The proximate composition for both PSH and CH is shown in Table 3.1. The results of proximate analysis indicated that moisture, lipids and ash content were different between PSH and CH. In Table 3.2, mean moisture/protein ratio and moisture/protein+ash ratio for both PSH and CH are listed. Both moisture/protein ratio and moisture/protein+ash ratio for PSH and CH had no significant differences. The proximate analysis for wet pink salmon and various parts has been reported in previous studies (Bechtel, 2003; Bower *et al.*, 2009a; Bower *et al.*, 2009b; Sathivel, 2005; Sathivel *et al.*, 2007). These authors pointed out that the heads had the most abundant lipid content compared to the other processing byproducts for pink salmon as well as other salmon species.

The proximate analysis of wet Pacific cod and parts has been previously investigated (Bechtel, 2003; Gordon and Roberts, 1977; Iwasaki and Harada, 1985; Shahidi *et al.*, 1991). The authors summarized that Pacific cod would store their lipids in their livers similarly to other members of the gadoid family that have been analyzed and that Pacific cod heads would contain only a low amount of lipids. The lipid content of the Pacific cod livers ranged from 30-50% depending on harvesting season, whereas proximate analysis of lipids in Pacific cod heads averaged around 1%. The proximate composition of DPSH and DCH are listed in Table 3.3.

In Table 3.3, higher than expected amount of ash and lower than expected amount of lipid for DCH were detected due to the bone sampling (i.e., uneven distribution of bone fragments) compared to the wet samples. The proximate composition changes within 180 days storage for DPSH and DCH are shown in Figures 3.3 and 3.4. The water activity of dried head samples did not change during the storage for up to 180 days (Table 3.3).

The small changes in proximate composition for DPSH demonstrated that protein and lipid changed over storage time, while the mean moisture and ash contents remained stable (Fig 3.3). While the mean protein content gradually increased, the mean lipid content decreased in DPSH over the 180 days of storage. Changes in the proximate composition of smoked herring showed a similar trend regarding changes to the protein and lipid contents during storage (Yoshikawa and Tamamoto, 1942). Ikegami *et al.*, (1971) concluded that the lipid oxidation in dried mackerel under ambient storage conditions resulted in a decrease of their nutritive value. Reduction in lipid content for DPSH might be due to the chemical degradation of some lipid fractions (Daramola *et al.*,

2007). These changes could be also attributed to oxidation of abundant polyunsaturated fatty acids (PUFA) in DPSH.

It was suggested that drying would accelerate the oxidation of lipids in fish (Aitken and Connell, 1979). These authors argued that carbonyl compounds from lipid oxidation could react with the alpha-amino group of lysine to complete a complex chemical reaction. Cutting (1962) pointed out that these reactions could be responsible for any darkening or toughening of the dehydrated fish during storage. Koizumi *et al.*, (1980) suggested that sablefish was more stable with respect to lipid oxidation and did not show a dramatic change in the lipid content during storage when compared to freeze-dried bluefin tuna. These researchers emphasized that heme proteins, known to catalyze oxidative rancidity, were more abundant in tuna than in sablefish.

Both the moisture/protein ratio and the moisture/protein+ash ratio suggested that moisture/protein ratio and moisture/protein+ash ratio in DPSH and DCH did not show any significant differences (Figures 3.5 and 3.6). Current USDA standards require products labeled as jerky have values for moisture/protein ratio of 0.75 or lower to ensure that pathogenic bacteria would not grow in the product (USDA, 2007).

3.3.3 Lipid Oxidation

Initial TBARS values for PSH averaged 2.4 ± 0.3 mg MA/kg, while the TBARS values of CH averaged at 1.5 ± 0.2 mg MA/kg. However, the TBARS values for frozen PSH averaged 6.5 ± 0.1 mg MA/kg after 60 days storage in a -40°C freezer, while TBARS values of wet frozen CH averaged at 2.5 ± 0.2 mg MA/kg after 60 days storage at the

same freezer. The low amounts of lipids and heme proteins in CH might be the major reason of lower oxidation in the frozen storage compared to PSH (MacLean and Castell, 1964). Changes in TBARS levels for DPSH and DCH are depicted in Fig 3.7. The PV of PSH averaged 12.7 ± 0.9 mequiv oxygen/kg of tissue, while the PV of wet CH averaged at 10.0 ± 1.6 mequiv oxygen/kg of tissue. In Fig 3.8, the PV changes for both DPSH and DCH are displayed. The FFA values for both PSH and CH averaged at 0.9 % oleic acid ± 0.1%. The FFA changes for both DPSH and DCH are shown in Fig 3.9.

The TBARS has been widely used to estimate the extent of lipid oxidation in various meat products (Shahidi, 1994). Increased levels of MA would be an indicator for severe coronary artery diseases (Jung *et al.*, 2004). The TBARS levels for DPSH and DCH showed significant increases over the 180 days of storage. However, the TBARS levels of DCH remained in the acceptable range, while the TBARS levels of DPSH had already risen when stored at -40°C for 60 days and exceeded the maximum level allowable for human consumption after drying. Oxygen freely entering the storage containers might contribute to the level of the oxidation. In canned pink salmon, the TBARS values declined with increasing temperature (Kong, 2007). The author concluded that the sealed packages or oxygen scavengers could limit the level of oxidation. These results indicated that DCH would have up to 180 days of shelf life under ambient storage conditions.

The mean PV for both DPSH and DCH peaked after 30 days storage and diminished to 20 mequiv oxygen/kg level at the end of the storage. Kong (2007) stated that the reduction in PV led to the progress of the TBA values.

The FFA results showed that the FFA values for DPSH were significantly higher than those for DCH and after 90 days, the FFA levels for DPSH became unacceptable. In contrast, the FFA levels for DCH stayed below the maximum acceptable limit of 8% throughout storage. These results were most likely due to the higher amount of lipids available in DPSH. Drying could activate the enzymes responsible for lipid hydrolysis (Hwang and Regenstein, 1996), phospholipase A and B were reported as important enzymes for lipid hydrolysis in fish. The FFA would oxidize more readily than esterified fatty acids (Labuza, 1971). Refsgaard *et al.*, (2000) noted that free fatty acid levels increased from 0.6 to 10% of total lipids in salmon fillets stored at -10°C for 7 months. These researchers concluded that the formation of FFA was due to enzymatic hydrolysis of the neutral lipids. In addition, cooking inactivated the lipolytic enzymes that cause the FFA formation in salmon tissue. Concerning the origin of FFA during frozen storage, Hardy (1980) reported that the probable source of the FFA formation was due to a measured decrease in the concentration of phospholipids over time. However, De Koning *et al.*, (1966) indicated that that FFA formation would occur due to hydrolysis of neutral lipids as well as phospholipids.

3.3.4 Microbiological Content

The total viable counts (TVC) for both PSH and CH averaged 5.2 Log CFU/g. Freezing reduced the TVC by 2 Log CFU/g after 14 days storage at -40°C for both CH and PSH. Tarr (1954) concluded that freezing would cause an initial decrease in numbers of viable bacteria. Dussault (1956) recommended that coliforms in fishery products could be reduced by rapid freezing at low temperatures. Himelbloom *et al.*, (1991) noticed that

Pacific cod fillets had microbial counts between 10^3/g and 10^6/g. These authors also suggested that washing whole salmon would reduce skin microbial counts from 10^3/cm^2 to 10^2/cm^2. Shewan (1954) revealed that icing herring fillets overnight reduced the initial microbial load by 2 Log CFU/g. Adu *et al.*, (1983) mentioned that the decrease in microbial counts for dried products might indicate that growth was being limited by a lack of moisture. Bello and Pigott (1980) pointed out that reduction in the water activity caused by drying could be an applicable option for controlling microorganisms in seafood products. Shewan (1954) and Tarr (1954) concluded that the maximum acceptable TVC for dehydrated fishery products would be 10^4 colony forming units per gram. Tarr (1954) reported that drying below 50°C might result in higher microbial degradation and thus a critical temperature for drying. No detectable colonies were found in DPSH, whereas the TVC for DCH were detected (Fig 3.10). This might be due to the lack of available water in DPSH for microorganisms (Martone *et al.*, 2005). Mold growth was observed in DCH three days after being dried at 20°C for 50 hours. The water activity levels were measured at $a_w = 0.7$ adequate for mold growth. No mold colonies were detected on either DCH or DPSH presumably because of the low water activity levels < 0.6.

3.3.5 FAMEs

The FAMEs results for PSH and DPSH and for CH and DCH are presented in Tables 3.4 and 3.5. The fatty acids comprising the fish oil extracted from the head samples are depicted in Fig 3.11 and Fig 3.12. In one gram of PSH oil, abundant docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were measured. However, the mean DHA and EPA concentrations in one gram of DPSH oil dropped after 90 days storage. This

occurred during ambient temperature (21°C) storage, conditions that also affected the TBARS values for DPSH. The reduction in DHA and EPA levels for DCH was lower compared to DPSH. This lower decline was possibly due to the reduced amount of lipids and their TBARS values in DCH compared to DPSH. Some researchers found relatively similar results both for Pacific cod and salmon oil (Addison *et al.,* 1968; Iverson *et al.,* 2002; Lapis, 2010). Simopolous *et al.*, (1999) recommended that adequate intake of EPA+DHA for adults would be 650 mg/day. The DHA and EPA are more abundant in salmon oils extracted from salmon heads than from salmon muscle. Based on USDA nutritional data, 100 g of fresh pink salmon contains 182 mg of EPA and 333 mg of DHA (USDA, 2011). During the dehydration and the storage of dried fish, the oxidation of PUFA was determined previously (Doe, 1998). The oxidative rancidity would be a problem in fish heads because a critical enzyme catalyzing this process, lipoxygenase, can be present in the blood, skin and gills of fish. Based on the results, the proportion decrease in polyunsaturated fatty acids (PUFA)/ saturated fatty acids (SAT) ratio for dried fish heads indicated a progressive oxidation in the products that correlated well with the TBARS results. Ozden (2005) mentioned that the total SAT and PUFA changes over storage time in seafood could be an important precursor for the quality.

3.3.6 Amino Acids

Alpha amino acids are the monomers of proteins and free amino acids are one of the main components of non-protein nitrogen (Ozden, 2005). During storage of chilled fish, the amino acids would alter due to muscle autolysis and microorganisms. Gudmundsson and Hafsteinsson (1997) suggested that air-drying likely cause more protein denaturation than

lyophilization (freeze-drying). The amino acid profiles of freeze-dried (day 0) and dried (180 days) CH and PSH showed a reduction in essential amino acids at the end of storage (Tables 3.6 and 3.7). These results are consistent with those reported for Alaskan seafood processing byproduct fish meals (Smiley *et al.*, 2003).

3.3.7 Minerals

The mineral analyses of both DCH and DPSH are listed in Tables 3.8 and 3.9. Most likely, the high level of bone in fish heads coupled with the fact that the mineral component of vertebrate bone mainly contains $CaPO_4$ (Marie *et al.*, 2001; Wu *et al.,* 2011) compared to the concentrations of other minerals. Marine organisms can accumulate heavy metals through directly from their food. In Alaska, low levels of heavy metals are found in fish largely a function of the age of the fish (Burger *et al.*, 2007). These metals can pass to human through the consumption of seafood and when their concentrations are high, heavy metals can cause health disorders (Burger and Gochfeld, 2005; Storelli *et al.,* 2006). Trace metals can accumulate in different fish tissues including the liver, gills and muscles (Romeo *et al.,* 1999). The concentrations of trace metal depend on the species, the age of the fish, behavior of the fish as well as the location where it was harvested. These results indicate that dried fish heads would be a reasonable source of micromineral nutrients including zinc and iron.

3.4 Conclusion

This study suggests that drying Pacific cod heads for sale in East African markets would be feasible. The results from this project showed that drying fish heads by controlling the

water activity and moisture content and consequently managing microbial safety would be a successful technique depending on the species. The PSH might have a shelf life of up to 60 days due to the high TBARS values. The high lipid content in PSH makes them vulnerable to oxidative rancidity. However, respectable volumes of the polyunsaturated fatty acids DHA and EPA can be extracted for further refining from PSH. This study showed that ambient storage of dried PSH would benefit from antioxidant glazing through reducing their susceptibility to lipid oxidation. Moreover, different packaging techniques including vacuum packaging might minimize rancidity by restricting the flow of oxygen. The DCH could become a commercially-viable food product in East Africa. Drying CH reduces potential storage costs, such as those encountered with frozen products. Transportation costs would also be reduced by decreased weight through the elimination of significant amounts of water. Application of existing technologies can help to determine the most lucrative options for each element of the byproduct stream.

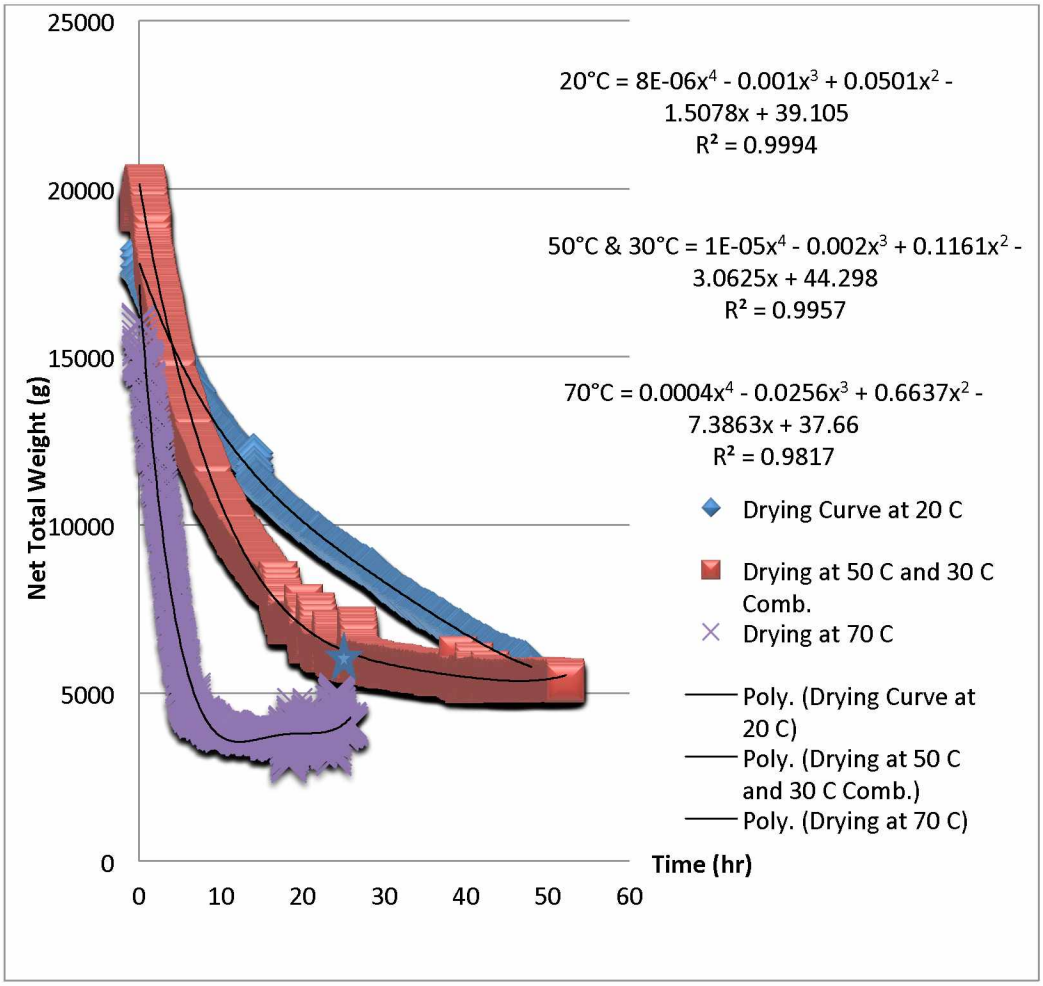

Figure 3.1 Drying curves for Pacific cod heads at various temperature and time (The blue star indicates starting point for 30°C drying).

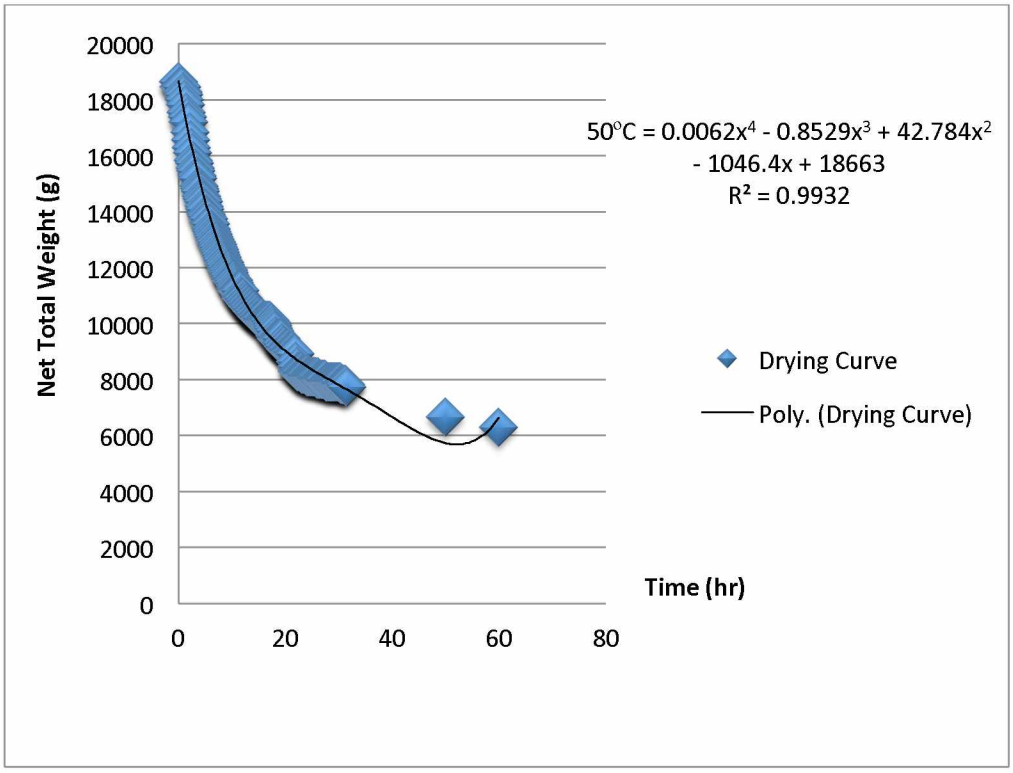

Figure 3.2 Drying curve for pink salmon heads at 50°C

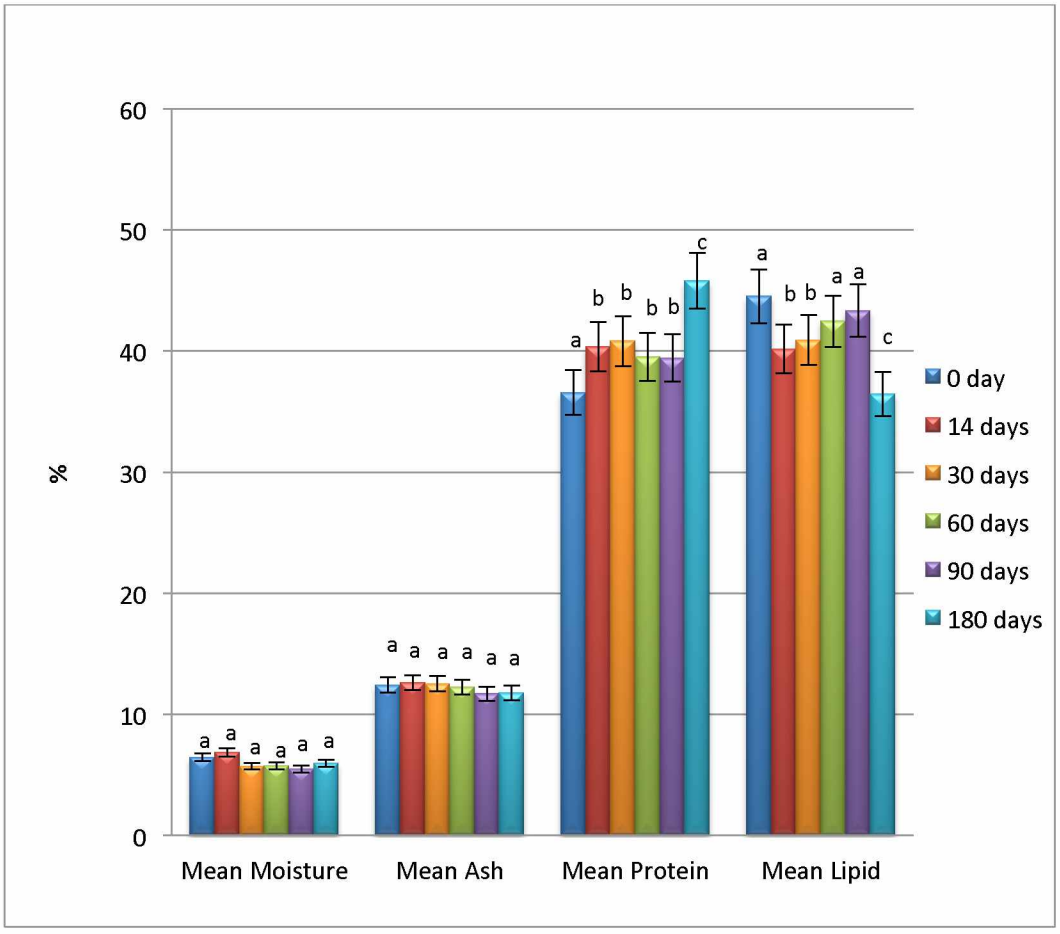

Figure 3.3 Proximate changes for dried pink salmon heads stored for up to 180 days

n=6; DPSH: Dried Pink Salmon Heads; Different letters within each category represent significant differences at $p < 0.05$.

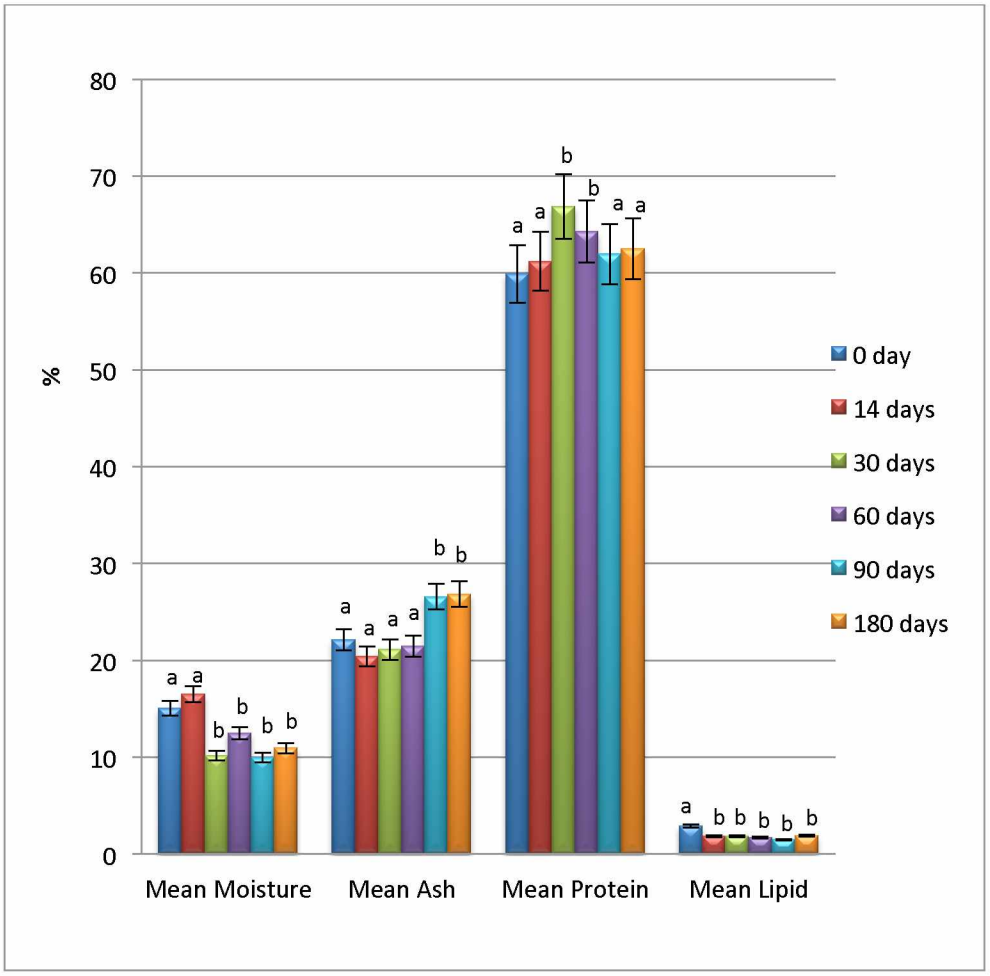

Figure 3.4 Proximate changes for dried Pacific cod heads stored for up to 180 days

n=6; DCH: Dried Pacific Cod Heads; Different letters represent significant differences at $p < 0.05$.

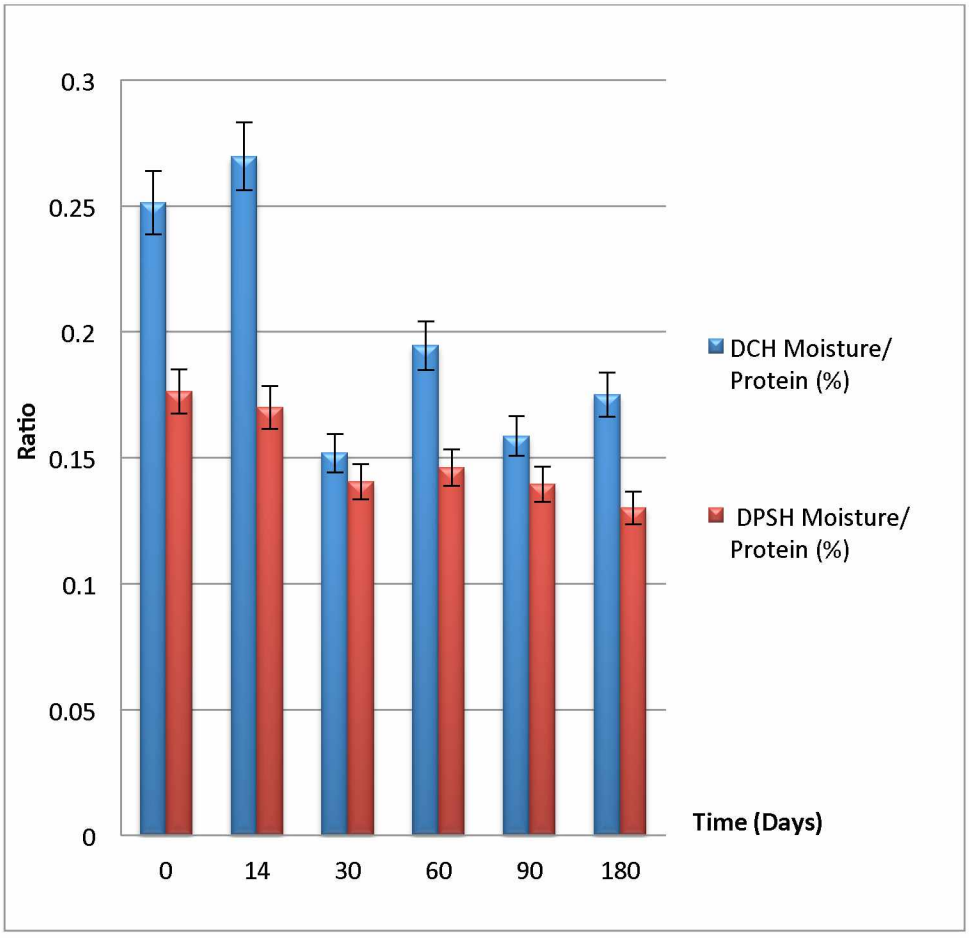

Figure 3.5 Moisture/protein ratio for dried pink salmon heads and dried Pacific cod heads stored for up to 180 days

n=6; DCH: Dried Pacific Cod Heads; DPSH: Dried Pink Salmon Heads.

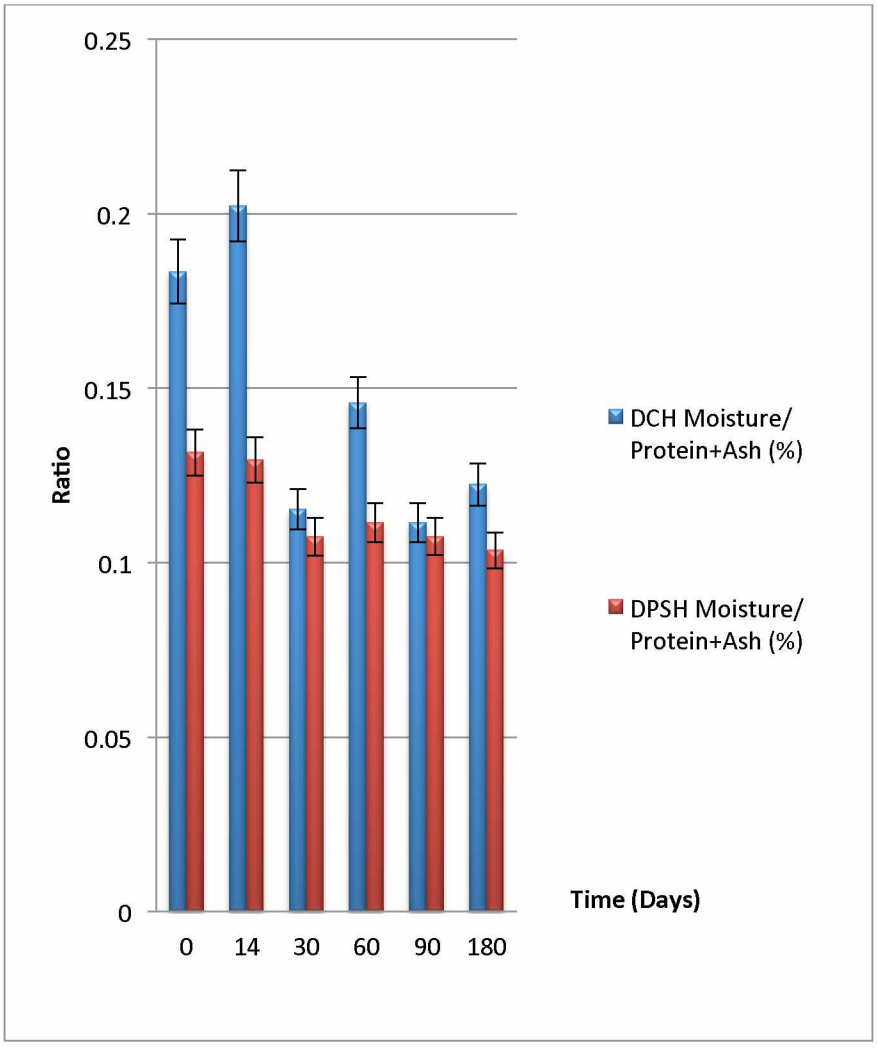

Figure 3.6 Moisture/protein+ash ratio for dried pink salmon heads and dried Pacific cod heads stored for up to 180 days

n=6; DCH: Dried Pacific Cod Heads; DPSH: Dried Pink Salmon Heads.

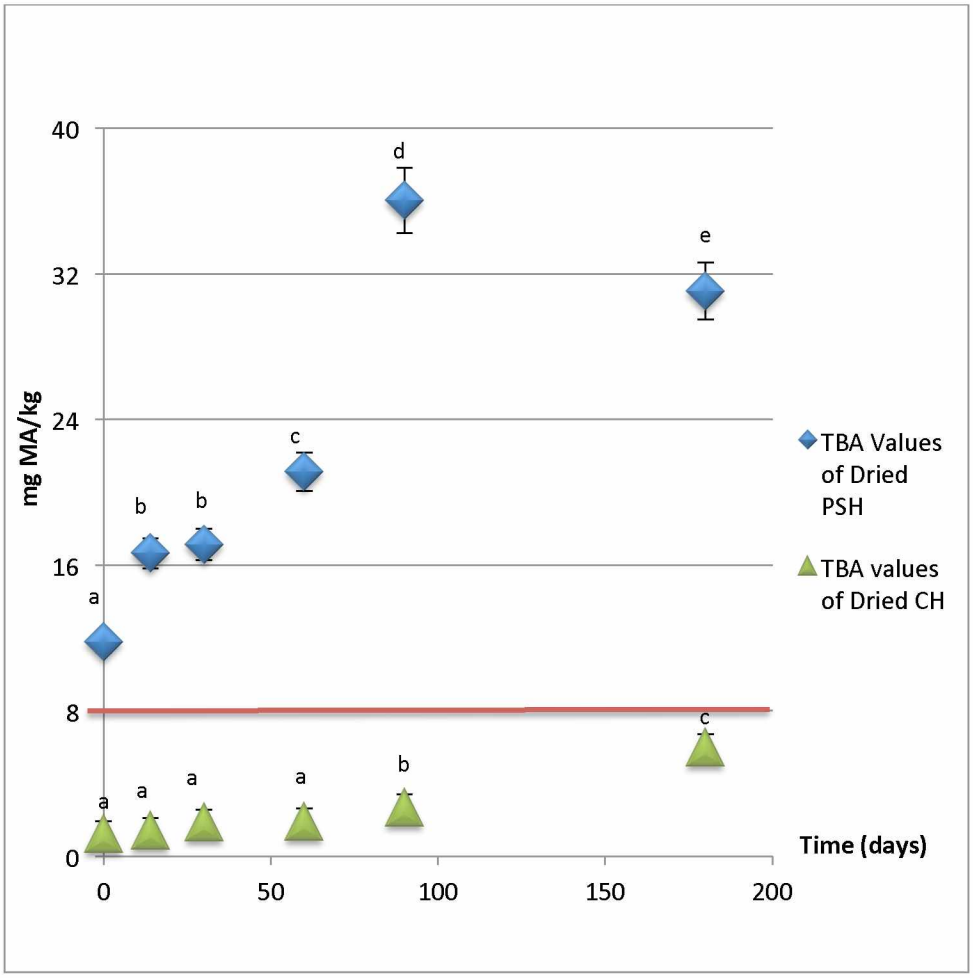

Figure 3.7 Thiobarbituric acid reactive substances levels for dried pink salmon heads and dried Pacific cod heads stored for up to 180 days

n=6; DPSH: Dried Pink Salmon Heads; DCH: Dried Pacific Cod Heads; MA: malonaldehyde; Different letters per species represent significant differences at $p < 0.05$. The red line is the maximum rancidity limitation for human consumption.

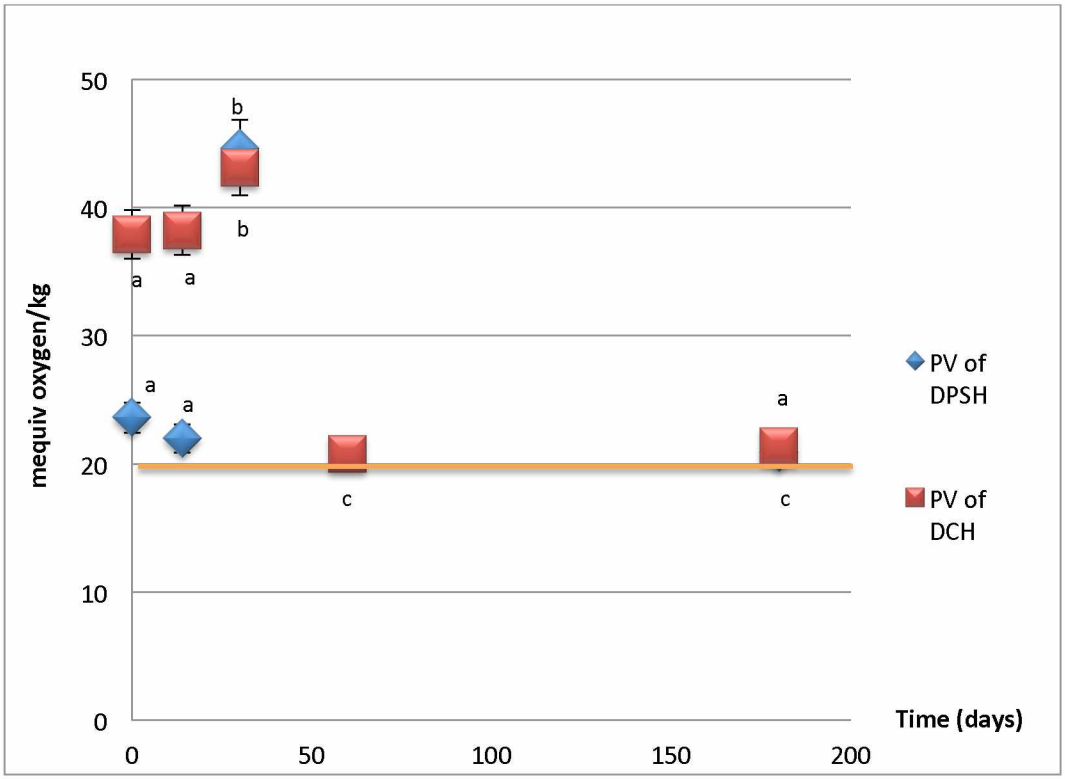

Figure 3.8 Peroxide values for dried pink salmon heads and dried Pacific cod heads stored for up to 180 days

n=6; DPSH: Dried Pink Salmon Heads; DCH: Dried Pacific Cod Heads; Different letters per species represent significant differences at $p < 0.05$. The orange line demonstrates the primary oxidation levels.

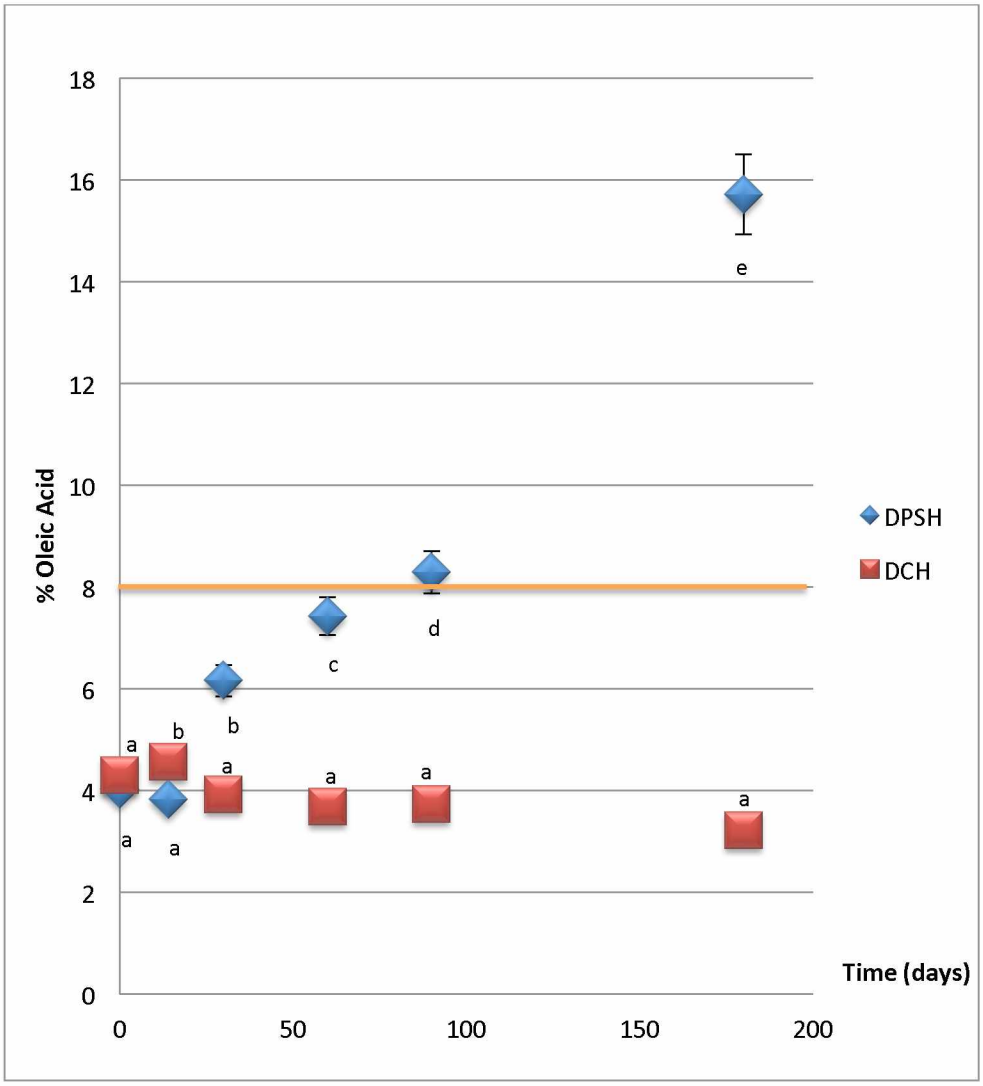

Figure 3.9 Free fatty acids levels for dried pink salmon heads and dried Pacific cod heads stored for up to 180 days

n=6; DPSH: Dried Pink Salmon Heads; DCH: Dried Pacific Cod Heads; Different letters per species represent significant differences at $p < 0.05$. The orange line is the maximum lipid hydrolysis limit for human consumption.

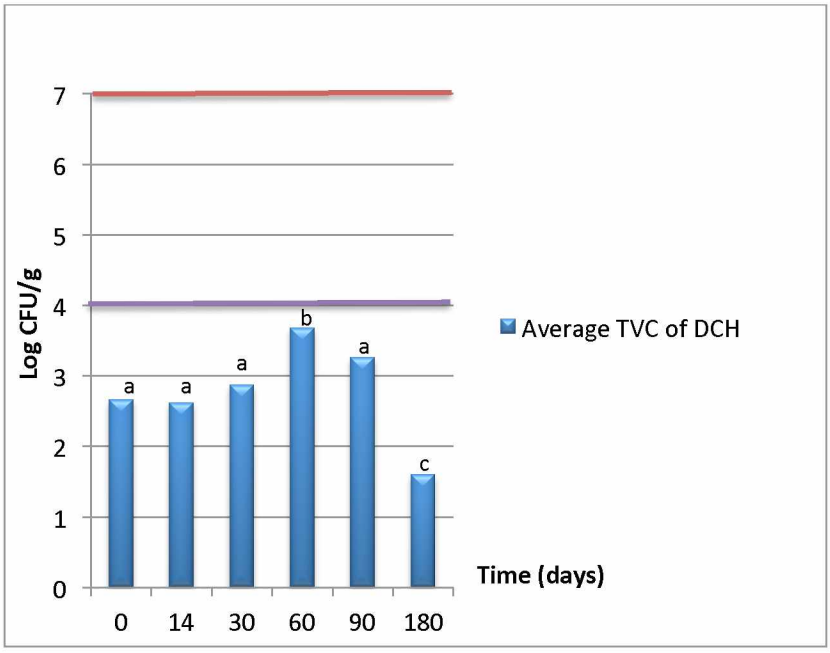

Figure 3.10 Total viable counts for dried Pacific cod heads stored for up to 180 days n=6; DCH: Dried Pacific Cod Heads; TVC: Total Viable Counts; Different letters represent significant differences at $p < 0.05$. The red line is the maximum acceptable TVC limit (frozen/fresh seafood). The purple line is the maximum acceptable TVC limit (dried seafoods).

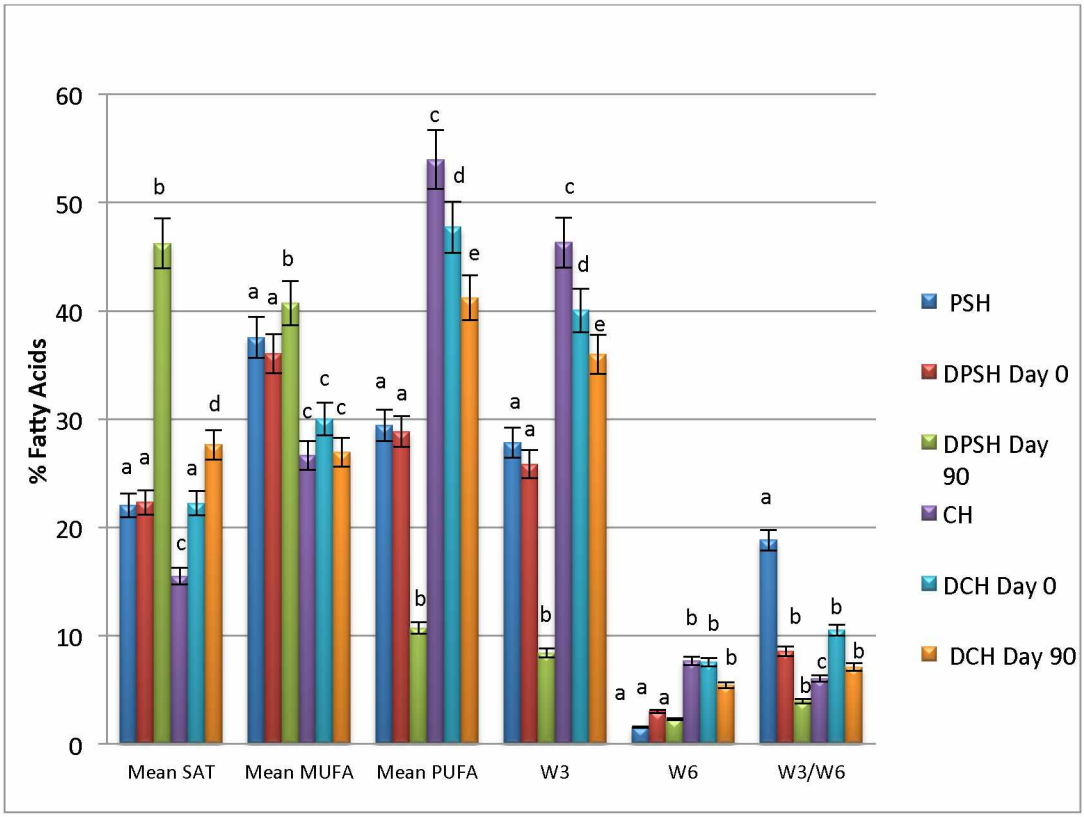

Figure 3.11 Fatty acids methyl esters levels of fish heads

n=6; PSH: Pink Salmon Heads; DPSH: Dried Pink Salmon Heads; CH: Pacific Cod Heads; DCH: Dried Pacific Cod Heads; SAT: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; Different letters within each group represent significant differences at $p < 0.05$.

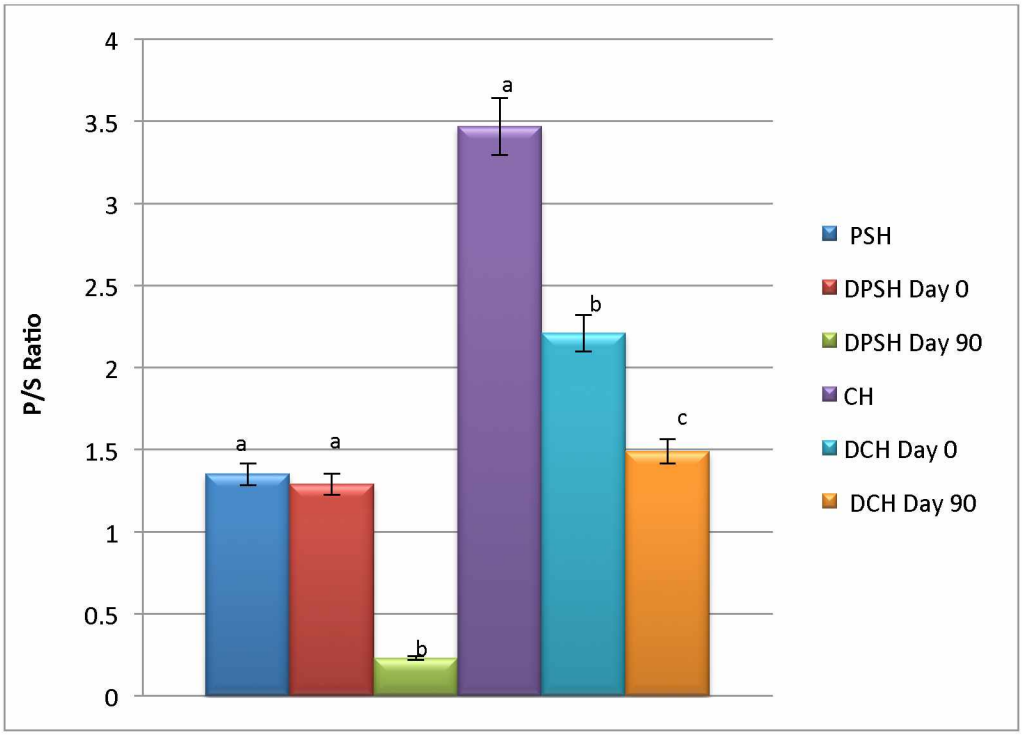

Figure 3.12 Polyunsaturated fatty acids/saturated fatty acids ratio of fish heads

n=6; PSH: Pink Salmon Heads; DPSH: Dried Pink Salmon Heads; CH: Pacific Cod Heads; DCH: Dried Pacific Cod Heads; P/S: Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters within a species represent significant differences at $p < 0.05$.

Table 3.1 Proximate analysis for pink salmon and Pacific cod heads

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
PSH	1.0^a	0.0	71.7^a	0.9	9.4^a	1.1	13.8^a	1.4	5.1^a	0.2
CH	1.0^a	0.0	81.0^b	1.3	1.2^b	0.1	15.5^a	1.2	2.3^b	0.6

n=6; PSH: Pink Salmon Heads; CH: Pacific Cod Heads; SD: Standard Deviation;

Different letters within column indicate statistical differences at $p < 0.05$.

Table 3.2 Moisture/protein ratio and moisture/protein+ash ratio for pink salmon and Pacific cod heads

Samples	Mean Moisture/Protein Ratio	SD	Mean Moisture/Protein+Ash Ratio	SD
PSH	5.2	0.5	3.8	0.2
CH	5.2	0.7	4.5	0.3

n=6; PSH: Pink Salmon Heads; CH: Pacific Cod Heads; SD: Standard Deviation.

Table 3.3 Proximate analysis for dried pink salmon heads and dried Pacific cod heads

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
DPSH	0.5^a	0.0	6.5^a	0.9	44.5^a	1.7	36.6^a	1.7	12.4^a	0.5
DCH	0.5^a	0.0	15.1^b	2.7	2.9^b	0.2	59.9^b	4.4	22.1^b	3.7

n=6; DPSH: Dried Pink Salmon Heads; DCH: Dried Pacific Cod Heads; SD: Standard Deviation; Different letters within column indicate statistical differences at $p < 0.05$.

Table 3.4 Fatty acid methyl esters for wet and dried pink salmon heads

FAMEs	Mean Area PSH (%)	SD	Mean Area (%) DPSH Day 0	SD	Mean Area (%) DPSH Day 90	SD
14:0	4.5[a]	0.5	5.3[b]	1.2	5.4[b]	1.2
14:1	0.9[a]	0.1	0.8[a]	0.3	0.8[a]	0.1
16:0	18.9[a]	2.6	15.1[b]	4.3	21.9[c]	2.3
16:1	1.0[a]	0.5	3.3[b]	0.8	6.0[c]	0.8
18:0	2.8[a]	0.8	1.1[b]	1.2	5.5[c]	0.9
18:1n9	12.2[a]	3.4	12.3[a]	3.3	22.7[b]	5.3
18:2n6	1.1[a]	0.2	1.6[a]	0.3	0.9[a]	0.2
20:5n3*	11.8[a]	1.5	4.5[b]	4.0	2.4[c]	0.5
22:6n3**	14.6[a]	1.6	7.7[b]	2.2	4.2[c]	0.7
24:1	0.8[a]	0.4	1.6[a]	0.6	0.9[a]	0.4
ΣSAT	26.2[a]	2.4	21.5[b]	1.5	32.8[b]	4.4
ΣMUFA	14.0[a]	2.9	17.2[b]	1.1	29.6[c]	4.4
ΣPUFA	27.5[a]	2.3	13.8[b]	1.9	7.5[c]	0.8
ΣP/S	1.1[a]	0.2	0.6[a]	1.7	0.2[b]	0.0
Σω3	26.4[a]	2.0	12.2[b]	2.5	6.6[c]	0.8
Σω6	1.1[a]	0.4	1.6[a]	1.5	0.9[a]	0.6
Σω3/ ω6	24.0[a]	5.5	7.6[b]	2.0	7.3[b]	1.4

n=6; PSH: Pink Salmon Heads; DPSH: Dried Pink Salmon Heads; SD: Standard deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$.* EPA and ** DHA.

Table 3.5 Fatty acid methyl esters for wet and dried Pacific cod heads

FAMEs	Mean Area CH (%)	SD	Mean Area DCH Day 0 (%)	SD	Mean Area DCH Day 90 (%)	SD
14:0	1.0[a]	0.6	0.8[a]	0.2	1.2[a]	0.9
16:0	9.8[a]	1.3	13.0[b]	1.4	13.6[b]	0.6
16:1	1.1[a]	0.3	1.8[a]	0.5	6.0[b]	0.8
18:0	1.7[a]	1.5	6.6[b]	1.2	4.7[c]	0.6
18:1n9	9.9[a]	3.7	17.9[b]	5.5	15.9[c]	2.1
18:2n6	1.7[a]	0.6	1.5[a]	0.1	1.6[a]	0.2
20:5n3*	13.7[a]	1.8	10.9[b]	2.5	9.5[c]	0.8
22:6n3**	31.4[a]	2.2	25.9[b]	2.5	24.9[b]	2.7
24:1	4.1[a]	1.7	2.0[b]	1.2	2.6[b]	0.6
ΣSAT	15.5[a]	0.7	22.2[b]	2.8	27.6[c]	0.8
ΣMUFA	15.1[a]	2.0	21.7[b]	8.0	24.5[a]	3.8
ΣPUFA	46.8[a]	1.0	38.3[b]	9.7	36.0[c]	3.8
ΣP/S	3.0[a]	0.9	1.7[b]	0.6	1.3[c]	0.2
Σω3	45.1[a]	1.4	36.8[b]	1.0	34.4[c]	1.9
Σω6	1.7[a]	0.8	1.5[a]	6.2	1.6[a]	1.5
Σω3/ ω6	26.5[a]	1.1	24.5[b]	1.6	21.5[c]	2.1

n=6; CH: Pacific Cod Heads; DCH: Dried Pacific Cod Heads; SD: Standard Deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$. * EPA and ** DHA.

Table 3.6 Amino acid profiles for freeze-dried (time 0) Pacific cod heads and air-dried Pacific cod heads stored for 180 days

Amino Acid (AA)	Freeze-Dried CH (wt/wt %)	SD	Dried CH (wt/wt %)	SD
Hydroxyproline (HYP)	0.6[a]	0.1	2.6[b]	0.7
Aspartic Acid (ASP)	10.5[a]	0.1	9.3[b]	0.5
Threonine (THR)	4.6[a]	0.0	4.0[a]	0.2
Serine (S)	4.6[a]	0.1	4.9[a]	0.2
Glutamic Acid (GLU)	15.9[a]	0.2	13.9[b]	0.6
Proline (PRO)	3.7[a]	0.2	6.4[b]	1.1
Glycine (GLY)	5.3[a]	0.3	10.9[b]	1.8
Alanine (ALA)	6.0[a]	0.0	6.9[a]	0.2
Valine (VAL)	5.2[a]	0.1	4.3[a]	0.3
Methionine (MET)	3.5[a]	0.0	3.0[a]	0.2
Isoleucine (ILE)	4.7[a]	0.1	3.7[b]	0.4
Leucine (LEU)	8.3[a]	0.1	6.5[b]	0.6
Tyrosine (TYR)	4.1[a]	0.1	3.0[b]	0.3
Phenylalanine (PHE)	4.3[a]	0.1	3.7[a]	0.3
Histidine (HIS)	2.2[a]	0.0	1.8[a]	0.1
Homolysine (HLYS)	0.2[a]	0.1	0.6[a]	0.1
Lysine (LYS)	9.2[a]	0.3	6.9[b]	0.8
Arginine (ARG)	7.1[a]	0.1	7.6[a]	0.2
Total EA	42.0[a]	0.2	34.0[b]	0.0
Total NEA	58.0[a]	0.1	66.0[b]	0.1
Total	100.0	0.1	100.0	0.1

n= 3; FDCH: Freeze-Dried Pacific Cod Heads (Time 0); DCH: Dried Pacific Cod Heads (time 180 days); SD: Standard Deviation; Total EA: Total Essential Amino Acids; Total NEA: Total Non-Essential Amino Acids; wt/ wt %: Weight/ Weight Percentage; Different letters in a row represent significant differences at $p < 0.05$.

Table 3.7 Amino acid profiles for freeze-dried (time 0) pink salmon heads and air-dried pink salmon heads stored for 180 days

Amino Acid (AA)	Freeze-Dried PSH (wt/wt %)	SD	Dried PSH (wt/wt %)	SD
Hydroxyproline (HYP)	3.1[a]	0.5	3.9[a]	0.2
Aspartic Acid (ASP)	9.1[a]	0.3	8.8[a]	0.3
Threonine (THR)	4.1[a]	0.2	3.8[a]	0.1
Serine (S)	4.4[a]	0.1	4.6[a]	0.1
Glutamic Acid (GLU)	13.3[a]	0.4	12.6[a]	0.3
Proline (PRO)	6.6[a]	0.8	7.8[b]	0.2
Glycine (GLY)	11.4[a]	1.1	13.3[b]	0.5
Alanine (ALA)	6.8[a]	0.1	7.0[a]	0.1
Valine (VAL)	4.3[a]	0.2	3.9[a]	0.1
Methionine (MET)	3.1[a]	0.1	2.9[a]	0.1
Isoleucine (ILE)	3.6[a]	0.2	3.1[a]	0.2
Leucine (LEU)	6.3[a]	0.4	6.2[a]	1.0
Tyrosine (TYR)	3.1[a]	0.1	2.7[a]	0.0
Phenylalanine (PHE)	3.8[a]	0.3	3.6[a]	0.1
Histidine (HIS)	2.1[a]	0.1	1.9[a]	0.1
Homolysine (HLYS)	0.9[a]	0.1	1.2[a]	0.1
Lysine (LYS)	6.7[a]	0.3	5.6[b]	0.3
Arginine (ARG)	7.3[a]	0.1	7.2[a]	0.1
Total EA	34.0[a]	0.2	31.0[b]	0.1
Total NEA	66.0[a]	0.1	69.0[b]	0.0
Total	100.0[a]	0.1	100.0[a]	0.1

n= 3; FDPSH: Freeze-Dried Pink Salmon Heads (Time 0); DPSH: Dried Pink Salmon Heads (time 180 days); SD: Standard Deviation; Total EA: Total Essential Amino Acids; Total NEA: Total Non-Essential Amino Acids; wt/ wt %: Weight/ Weight Percentage; Different letters in a row represent significant differences at $p < 0.05$.

Table 3.8 Minerals in dried Pacific cod heads stored for 180 days

Mineral	DCH	SD
P (%)	3.8	0.8
K (%)	0.8	0.1
Ca (%)	8.2	1.7
Mg (%)	0.2	0.0
Cu (ppm)	1.2	0.1
Zn (ppm)	62.2	8.2
Mn (ppm)	13.5	4.3
Fe (ppm)	4.7	1.2
Cd (ppm)	0.0	0.0
Ni (ppm)	0.4	0.2
Pb (ppm)	0.3	0.2
As (ppm)	5.8	3.2
Sr (ppm)	630.3	120.3

n= 6; DCH: Dried Pacific Cod Heads; SD: Standard Deviation.

Table 3.9 Minerals in dried pink salmon heads stored for 180 days

Mineral	DPSH	SD
P (%)	1.8	0.3
K (%)	0.5	0.0
Ca (%)	6.0	2.6
Mg (%)	0.1	0.0
Cu (ppm)	1.2	0.2
Zn (ppm)	71.3	7.8
Mn (ppm)	1.1	0.3
Fe (ppm)	22.8	5.3
Cd (ppm)	0.0	0.0
Ni (ppm)	0.2	0.1
Pb (ppm)	0.1	0.1
As (ppm)	1.2	0.4
Sr (ppm)	177.0	37.9

n= 12; DPSH: Dried Pink Salmon Heads; SD: Standard Deviation.

3.5 References

Abbas K.A., Saleh A.M., Mohammed A. and Lasekan O., 2009. The relationship between water activity and fish spoilage during cold storage: A review. J. Food Agr. Env. 7(3&4): 86-90.

Addison R.F., Ackman R.G. and Hingley J., 1968. Distribution of fatty acids in cod flesh lipids. J. Fish Res. BD. Canada. 25(10): 2083-2090.

Adu G.A., Babbitt K. and Crawford D.L., 1983. Effects of washing on the nutritional and quality characteristics of dried minced rockfish flesh. J. Food Sci. 48: 1053-1060.

Aitken A. and Connell J.J., 1979. In: Effects of Heating on Foodstuffs. Priestley R.J. (Ed.). Applied Science Publishers. London. 219-254.

AOAC, 2005. Official methods of analysis of AOAC International. 18th edition. Association of Official Analytical Chemists International, Arlington. VA.

AOCS, 1998. Official methods and recommended practices of the American Oil Chemists' Society (5th Edition). Champaign, Illinois, Washington, DC.

Arason S., 2001. Drying of fish and utilization of geothermal energy-The Icelandic experience. Keynote lectures. 1st Nordic drying conference. Trondheim.

Bechtel P.J., 2003. Utilization of Alaska's seafood processing byproducts. In Advances in seafood byproducts. Bechtel P.J. (Ed.). Alaska Sea Grant College Program, University of Alaska Fairbanks. pp. 105-119.

Bello R.A. and Pigott G.M., 1980. Dried fish patties: Storage stability and economic considerations. J. Food Pro. Pres. 4: 247-260.

Bimbo P.A., 2009. Alaska seafood byproducts: Potential products, markets and competing products. Report for Alaska Fisheries Development Foundation. Anchorage, AK. pp. 227.

Bower C.K., Hietala K.A., Oliveira A.C.M. and Wu T.H., 2009a. Stabilizing oils from smoked pink salmon (*Oncorhynchus gorbuscha*). J. Food Sci. 74 (3): 248-257.

Bower C.K., Malemute C. and Bechtel P.J., 2009b. Endogenous protease activity in byproducts of pink salmon (*Oncorhynchus gorbuscha*). J. Food Bio. 35: 628-637.

Burger J. and Gochfeld M., 2005. Heavy metals in commercial fish in New Jersey. Env. Res. 99: 403-412.

Burger J., Gochfeld M., Jeitner C., Burke S., Stamm T., Snigaroff R., Snigaroff D., Patrick R. and Weston J., 2007. Mercury levels and potential risk from subsistence foods from the Aleutians. Sci. Tot. Env. 384: 93-105.

Burt J.R., 1988. Fish smoking and drying: The effect of smoking and drying on the nutritional properties of fish. Burt J.R. (Ed.). Elsevier Publishers. UK. pp. 166.

Connell J.J., 1995. Control of fish quality. 4th Edition. Oxford: Fishing new books. pp. 241.

Cutting C.L., 1962. The influence of drying, salting and smoking on the nutritive value of fish. In: Fish in nutrition. Heen E. and Kreuzer R. (Eds.). Fishing News. London. pp. 161-179.

Daramola J.A., Fasakin E.A. and Adeparusi E.O., 2007. Changes in physiochemical and sensory characteristics of smoke-dried fish species stored at ambient temperature. African J. Food Agr. Nutr. Dev. 7(6): 1-16.

De Koning A.J., 1966. Phospholipids of marine origin. The hake (*Merlucius capensis*). J. Sci. Food Agr. 17: 112-117.

Doe E.P., 1998. Fish drying and smoking production and quality. Doe E.P. (Ed.). Technomic Publishing, PA. pp. 250.

Dussault H.P., 1956. Effect of freezing on coliform bacteria and method of detection in frozen fish fillets and blocks. Fish. Res. Br. Can. 65: 12-14.

Fennema R.O., 1996. Food chemistry. 3rd Edition. Fennema R.O. (Ed.). Marcel Dekker, New York. pp. 1069.

Fiskeriforskning, 2004. Focus on fisheries research: Use your head. Handout no.1. Tromso, Norway.

Freeman D.W. and Hearnsberger D.O., 1994. Rancidity in selected sites of frozen catfish fillets. J. Food Sci. 69(1): 60-63.

Gordon D.T. and Roberts G.L., 1977. Mineral and proximate composition of Pacific coast fish. J. Agr. Food Chem. 25(6): 1262-1267.

Gudmundsson M. and Hafsteinsson H., 1997. Gelatin from cod skins affected by chemical treatments. J. Food Sci. 62(1): 37-47.

Hardy R., 1980. Fish lipids. Part 2. In: Advances in Fish Science and Technology. Connell J.J. (Ed.). Fishing News. London. pp. 103-111.

Himelbloom B.H., Brown E.K. and Lee J.S., 1991. Microorganisms on commercially processed Alaskan finfish. J. Food Sci. 56(5): 1279-1281.

Hwang K.T. and Regenstein J.M., 1996. Lipid hydrolysis and oxidation of mackerel (*Scomber scombrus*) mince. J. Aq. Food Prod. Tech. 5: 17-27.

ICMSF, 1978. Microorganisms in foods. Vol. 1. The International Commission on Microbiological Specifications for Foods. Toronto, ON, Canada. University of Toronto Press. pp. 343.

Ikegami Y., Takai Y. and Shibuya K., 1971. Nutritive value of frozen food VIII. Nutritive value of frozen squid and mackerel. Report Inst. Nut. pp. 73-78.

Iverson S.J., Frost K.J. and Lang S.L.C., 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: Factors contributing to among and within species variability. Mar. Ecol. Prog. Ser. 241: 161-181.

Iwasaki M. and Harada R., 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. J. Food Sci. 50: 1585-1587.

Jung H.H., Choi D.H. and Lee S.H., 2004. Serum malondialdehyde and coronary artery disease in hemodialysis patients. Am. J. Neph. 24(5): 537-542.

Kingsly R.P., Goyal R.K., Manikantan M.R. and Ilyas S.M., 2007. Effects of pretreatments and drying air temperature on drying behavior of peach slice. Inter. J. Food Sci. 42: 65-69.

Koizumi C., Terashima H., Wada S. and Nonaka J., 1980. Lipid oxidation of salted freeze-dried meats at different equilibrium relative humidities. Bull. Japan Soc. Sci. Fisheries. 46: 871-877.

Kong F., 2007. Kinetics of salmon (*Oncorhynchus gorbuscha*) quality changes during thermal processing. Dissertation. Washington State University, Department of Biological Systems Engineering. Pullman, WA. pp. 186.

Labuza T.P., 1971. Kinetics of lipid oxidation in foods. In: CRC Critical Reviews in Food Technology. CRC Press. pp. 355-405.

Lapis T., 2010. Improving the sensorial and nutritional attributes of canned Alaska pink salmon (*Oncorhynchus gorbuscha*) with salmon oil. M.Sc. Thesis. University of Alaska Fairbanks. pp. 121.

Lemon D.W., 1975. An improved TBA test for rancidity. Environment Canada. Fisheries and Marine Service. New Series Circular. 51: 52-55. Halifax, Nova Scotia.

MacLean J. and Castell C.H., 1964. Rancidity in lean fish muscle. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. J. Fish Res. Bd. Canada. 21(6): 1345-1359.

Marie P.J., Ammann P., Boivin G. and Rey C., 2001. Mechanisms of action and therapeutic potential of strontium in bone. Calcif. Tissue Int. 69(3): 121-129.

Martone C.B., Borla O.P. and Sanchez J.J., 2005. Fishery byproduct as a nutrient source for bacteria and archaea growth media. Bioresource Tec. 96(3): 383-387.

Maxwell J.R. and Marmer N.W., 1983. Methods: Systematic protocol for the accumulation of fatty acid data from multiple tissue samples: Tissue handling, lipid extraction and class separation, and capillary gas chromatographic analysis. Lipids. 18(7): 453-459.

Messer J.W., Peeler J.T. and Gilchrist J.E., 1984. Aerobic plate count. Chapter 4. In: Bacteriological Analytical Manual. Food and Drug Administration. Association of Official Analytical Chemists. Arlington, VA.

Mol S., 2005. Preparation and the shelf life assessment of ready-to-eat fish soup. Eur. Food Res. Technol. 220: 305-308.

Ozden O., 2005. Changes in amino acid and fatty acid composition during shelf life of marinated fish. J Sci. Food Agr. 85: 2015-2020.

Refsgaard H.H.F., Brockhoff P.M.B. and Jensen B., 2000. Free polyunsaturated fatty acids cause taste deterioration of salmon during frozen storage. J. Agr. Food Chem. 48: 3280-3285.

Richards M.P., Nelson N.M., Kristinsson H.G., Mony S.S.J., Petty H.T. and Oliveira A.C.M., 2007. Effects of fish heme protein structure and lipid substrate composition on hemoglobin-mediated lipid oxidation. J. Agr. Food Chem. 55: 3643-3654.

Romeo M., Siau Y., Sidoumou Z. and Gnassia-Barelli M., 1999. Heavy metal distribution in different fish species from the Mauritania coast. Sci. Total Env. 232: 169-175.

Sathivel S., 2005. Thermal and flow properties of oils from salmon heads. J. American Oil Chem. Soc. 82(2): 147-152.

Sathivel S., Liu Q., Huang J. and Prinyawiwatkul W., 2007. The influence of chitosan glazing on the quality of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. J. Food Eng. 83: 366-373.

Schormuller J., 1968. Handbuch der lebensmittelchemie (Band III/2). Springer Verlag. Berlin Heidelberg. New York. Handbook of Food Chem. pp. 1341-1397.

Shahidi F., 1994. Proteins from seafood processing discards. In: Seafood proteins. Sikorski ZE., Pan BS. and Shahidi F. (Eds.). Chapman and Hall. New York. pp. 171-193.

Shahidi F., Naczk M., Pegg R.B. and Synowiecki J., 1991. Chemical composition and nutritional value of processing discards of cod (*Gadus morhua*). Food Chem. 42: 145-151.

Shewan J.M., 1954. The bacteriology of dehydrated fish. III. Observations and experiments made during small scale commercial production. J Hyg. 52(2): 247-252.

Shiau C.Y. and Chai T., 1990. Characterization of oyster shucking liquid wastes and their utilization as oyster soup. J. Food Sci. 55(2): 374-378.

Simopoulos A.P., Leaf A. and Jr. Salem N., 1999. Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. ISSFAL Newslett. 6(2): 14-16.

Smiley S., Babbitt J., Divakaran S., Forster I. and Oliveira A.C.M., 2003. Analysis of groundfish meals made in Alaska. In: Advances in seafood byproducts: 2002 conference proceedings. Bechtel P.J. (Ed.). Alaska Sea Grant College Program. University of Alaska Fairbanks. pp. 431-454.

Storelli M.M., Barone G., Storelli A. and Marcotrigiano G.O., 2006. Trace metals in tissues of Mugilids (*Mugil auratus*, *Mugil capito* and *Mugil labrosus*) from the Mediterranean Sea. Bull. Env. Cont. Tox. 77: 43-50.

Tarr H.L.A., 1954. Microbiological deterioration of fish post mortem, its detection and control. Microbiol. Mol. Biol. Rev. 18(1): 1-15.

USDA, 2007. Compliance guideline for meat and poultry jerky produced by small and very small plants. The United States Department of Agriculture. pp. 1-10. Available at: www.fsis.usda.gov/pdf/compliance_guideline_jerky.pdf.

USDA, 2011. Online nutrient data laboratory. The United States Department of Agriculture. Available at: www.nal.usda.gov/fnic/foodcomp/search.

Wein E.E., 1995. Evaluating food use by Canadian aboriginal people. Can. J. Physio. Pharma. 73: 759-764.

Wu T.H., Nigg J.D., Stine J.J. and Bechtel P.J., 2011. Nutritional and chemical composition of by-product fractions produced from wet reduction of individual red salmon (*Oncorhynchus nerka*) heads and viscera. J. Aq. Food Product Tech. 20: 183-195.

Yoshikawa Y. and Tamamoto I., 1942. Change in chemical compositions of fish during smoking process-I. Cold smoked herring. J. Fisheries. 50: 6-11.

Chapter 4. Antioxidant Effects on Frozen and Dried Pink Salmon Heads

4.1 Introduction

The use of natural antioxidants can be effective for limiting rancidity (Frankel, 1993; Lopez *et al.*, 2005; Shahidi and Botta, 1994). The objectives of this study were: to determine the biochemical and microbiological differences, if any, between wet early and late run pink salmon heads (ERPSH and LRPSH), to determine the moisture content and water activity of dried heads for pink salmon heads (PSH), to determine the effect of antioxidant treatment (+AO) on oxidation levels of frozen ERPSH and LRPSH during storage for up to 60 days at frozen storage (-40°C) for up to 60 days and to determine the effect of antioxidant on drying LRPSH when stored for up to 60 days at ambient temperature. In lipid oxidation, oxygen has the greatest responsibility compared to enzymatic oxidation (McClements and Decker, 2000). Combinations of natural extracts of rosemary and tocopherol synergistically delay oxidation for fatty fish species (Serdaroglu and Felekoglu, 2005; Wada and Fang, 1992).

4.2 Materials and Methods

4.2.1 Fish Head Processing

The ERPSH and LRPSH were freshly collected from a local processor in Kodiak, AK. The gills were manually removed and the heads split prior to drying. Three drying operations at 50°C for up to 72 hours (sampling times: 24, 36, 48, 55 and 72 hours) were conducted for ERPSH to obtain the correlation between moisture and water activity, as well as the drying coefficienct (DC %) at 50°C. Air velocity was recorded using a mini

thermo-anemometer (Extech Instruments, 45158, China). The antioxidant (Duralox MAN-5 NS, Kalsec Inc., Kalamazoo, MI) was mixed with 60°C water to produce a solution with a final 2% (v/v) concentration producing a bright yellow color. Untreated ERPSH and LRPSH served as controls, while ERPSH+AO and LRPSH+AO (dipped into 2% v/v antioxidant) were the experimental materials. The LRPSH and LRPSH+AO were dried for 55 hours to produce the target weight assuming a 10% moisture content. A total of 12 ERPSH and LRPSH (6 control + 6 antioxidant dipped) replicates were prepared for each biochemical and microbiological analysis.

4.2.2 Glazing Uptake

The ERPSH and LRPSH were dipped into the antioxidant solution for 2 minutes, let stand and dripped again for 1 minute and then frozen for 24 hours at -40°C. The percent glazing uptake was determined based on the Sathivel *et al.*, (2007) method by calculating the differences in weight.

4.2.3 Proximate Analysis

The proximate composition was determined for both wet and dried head samples. The moisture and ash contents were measured by AOAC methods 952.08 and 938.08 (AOAC, 2005). Protein analysis was carried out using an automated protein analyzer (Elementar Rapid N III, Hanau, Germany). The protein content was calculated by multiplying the percent nitrogen value by 6.25. The lipid content of wet and dried head samples was determined using an Accelerated Solvent Extraction (ASE Model 200, Dionex, Sunnyvale, CA).

4.2.4 Water Activity

The water activity levels (expressed as a_w) for wet and dried head samples were determined at room temperature using an Aqualab water activity meter (Model Series 3TE, Decagon Devices Inc., Pullman, WA).

4.2.5 TBARS Analysis

Thiobarbituric Acid Reactive Substances (TBARS) analysis was conducted to measure secondary oxidation levels (Lemon, 1975). The data were expressed as the concentration of malonaldehyde (MA) in the samples as mg MA/kg. The TBARS values below 6 mg MA/kg of fish tissue are considered as acceptable in terms of food safety and quality (Freeman and Hearnsberger, 1994). The maximum allowable MA content in human food is 8 mg MA/kg (Schormuller, 1968).

4.2.6 FFA Analysis

The concentration of Free Fatty Acids (FFA) can be used as a measure of lipid autolysis (Doe, 1998). It was determined according to AOCS method number Ca 5a-40 (AOCS, 1998). The FFA values were reported as % oleic acid for extracted lipid weight. The 3.0 % FFA is an acceptable limit for high quality fish oils (Bimbo, 2009). The maximum FFA acceptable levels for fish oils are between 7 and 8 %.

4.2.7 FAMEs Analysis

Fatty Acid Methyl Esters (FAMEs) were prepared using a modification of the Maxwell and Marmer method (1983). Gas chromatography (GC) was operated using a GC model 7890A (Agilent, Santa Clara, CA) fitted with a FAMEWAX^{TM} 30 m x 0.32 mm x 0.25

μm, GC column (Restek, Bellefonte, PA). The data were collected and analyzed using the GC ChemStation program (Ver E.02.00.493, Agilent, Santa Clara, CA). Helium was the carrier gas running at a velocity of 64 cm/sec. Injector and detector temperatures were held at 250°C and 280°C, respectively. A split injection mode (50:1 split ratio) was used. The oven temperature was increased from 50°C to 220°C at a rate of 5°C/minutes and held at 220°C for 2 additional minutes for a total running time of 36 minutes. An autosampler performed the GC injection of both standards and samples. The injection volume was 1μL. Samples were identified by comparing retention times to those of commercially available standards. The standards employed were: Supelco 37, PUFA # 1, PUFA #3 and Pacific cod liver oil from Supelco (Bellefonte, PA). The data were expressed as percent of total integrated area.

4.2.8 Microbiological Analysis

Five grams of the wet samples were weighed into a filter bag and filled with sterilized 0.1% peptone water up to fifty grams total weight. The samples were homogenized by using a stomacher (IUL Instruments, Barcelona, Spain) for two minutes. Aerobic plate counts were determined by spread plating onto pre-poured Bacto Plate Count Agar medium with 0.5% salt (Difco Laboratories, Detroit, MI) in triplicates. Plates were incubated at 25°C for 72 hours to evaluate the initial microbial load of wet samples and count bacteria (Messer *et al.*, 1984).

Two grams of the dried samples were weighed into a filter bag and filled with sterilized 0.1% peptone water up to twenty grams total weight. Total plate count was determined

using the same methods as wet samples. Sterilized Potato Dextrose Agar medium (Difco Laboratories, Detroit, MI), reduced to pH 3.5 with a 10% tartaric acid solution was used to enumerate mold growth on the dried samples in triplicates. Color determination sticks (EM Science, Darmstadt, Germany) were used to ensure the pH requirements for agar media. These plates were incubated at 35°C for 48 hours to count the bacteria and molds from the dried samples (Messer *et al.*, 1984). The acceptable limit for mold growth was set at zero in these experiments. The maximum aerobic plate count limit for fresh and frozen fish is 7 Log Colony Forming Units (CFU) /g as employed by International Commission on Microbiological Specifications for Foods (ICMSF, 1978).

4.2.9 Statistical Analysis

Statistica version 9.0 (Tulsa, OK) was used for determination of means for all measured parameters. An ANOVA test with Tukey HSD test was used for evaluating significant differences ($p < 0.05$).

4.3 Results and Discussion

4.3.1 Drying of Early Run Pink Salmon Heads

The moisture and water activity were recorded during air-drying at 50°C (24, 36, 48, 55 and 72 hours) in six replicates. In Fig 4.1, the moisture sorption, the relationship between water content and equilibrium humidity, of early run pink salmon heads is shown. Air velocity was recorded at 6.3 m/s throughout drying. Figure 4.2 shows the drying curves for three drying experiments. In Figure 4.2, the noises for weight changes during drying were due to the sensitive weight scale recording. Figure 4.3 shows the drying coefficient

(% DC). The drying trials demonstrated that 10% of moisture content in dried PSH was the critical limit required to reduce water activity below 0.6. This level was achieved after 55 hours at 50°C. The polynomial equations associated with the figure may prove useful for engineering purposes.

4.3.2 Glazing Uptake

The uptake of glazing, (reported as % uptake) for ERPSH and LRPSH was determined as 2.5% ± 0.6% and 2.4% ± 0.5%. Inhibitors in the antioxidant and their concentrations (Duralox MAN-5, Kalsec, Kalamazoo, MI) are presented in Fig 4.4.

4.3.3 Proximate Analysis of Wet Fish Heads

The proximate compositions of ERPSH and LRPSH (+AO) are reported in Tables 4.1 and 4.2. The moisture/protein ratio and the moisture/protein+ash ratio of ERPSH and LRPSH with/without antioxidant dipping (±AO) are reported in Table 4.3. The proximate analysis of the wet samples suggested that ERPSH were higher in lipids and lower in moisture and protein compared with LRPSH. This was likely due to the changes associated with spawning (Reid *et al.,* 1993).

4.3.4 Lipid Oxidation of Wet Fish Heads

The FFA and TBARS values for ERPSH and LRPSH ±AO are reported in Table 4.4 and Fig 4.5. No significant differences for mean FFA values in ERPSH and LRPSH±AO were found. However, the antioxidant dipping for pink salmon heads delayed lipid oxidation ($p < 0.05$). The results suggested that pink salmon heads would have only 60 days of shelf life at frozen storage conditions (-40°C) unless antioxidant glazing is

applied. High antioxidant activities of rosemary were previously observed in fish oil emulsions (Frankel and Huang, 1996). The mixture of alpha-tocopherol (Vitamin E) and rosemary extract showed synergistic effect on rancidity including sardine oil and frozen crushed fish meat (Wada and Fang, 1992), horse mackerel mince (Vareltzis *et al.,* 1997) and frozen sardine mince (Serdaroglu and Felekoglu, 2005) up to three months.

4.3.5 FAMEs in Wet Fish Heads

The FAMEs results of ERPSH and LRPSH ±AO are listed in Tables 4.5 and 4.6. The antioxidant dipping did not change the ratio of polyunsaturated fatty acids in ERPSH and LRPSH. The results suggested that LRPSH had slightly lower total omega-3 fatty acids compared to ERPSH. The antioxidant treatment allowed omega-3 fatty acids concentrations to remain higher in both ERPSH and LRPSH ($p < 0.05$).

4.3.6 Microbiological Content of Wet Fish Heads

Mold was absent in ERPSH. However, one replicate out of six for LRPSH averaged 3.5 Log CFU/g for mold. The mean TVC for ERPSH were 2.8 Log CFU/g, whereas the TVC for LRPSH were 3.2 Log CFU/g after frozen storage at -40°C for 14 days. The TVC for ERPSH and LRPSH glazed with the antioxidant solution showed no significant differences with 4.1 and 3.9 Log CFU/g, respectively after frozen storage at -40°C for 14 days. A 1- Log increase in ERPSH and LRPSH treated with antioxidant might be due to the antioxidant preparation conditions (Marriott and Gravani, 2006). Nevertheless, the TVC for all wet samples were below the established quality level of 7 Log CFU/g.

4.3.7 Drying of Late Run Pink Salmon Heads

The 50°C drying temperature was recorded for 55 hours for LRPSH±AO. Moisture/Solid Ratios for LRPSH±AO and drying curves for LRPSH±AO are shown in Figs 4.6 and 4.7. In Fig 4.6, the purple line on the y- axis at 0.1 suggests the target point for moisture content (10%) in LRPSH±AO. In Fig 4.7, the noises for weight changes during drying were due to the sensitive weight scale recording. The polynomial equations are included shown for engineering purposes.

4.3.8 Proximate Analysis

Changes in proximate analysis for DPSH±AO (dried late run pink salmon heads with/without antioxidant treatment) stored for up to 60 days are shown in Fig 4.8. The lipid and protein contents of DPSH changed during storage ($p < 0.05$). The antioxidant treatment resulted in higher lipid content during storage than was observed in DPSH without antioxidant treatment. The mean moisture/protein ratio and the moisture/protein+ash ratio for DPSH±AO are shown in Fig 4.9. Antioxidant treatment did not change significantly both moisture/protein and moisture/protein+ash ratios.

4.3.9 Lipid Oxidation

The TBARS values for DPSH±AO stored for up to 60 days are presented in Fig 4.10. DPSH had higher levels of oxidation than the maximum limit for consumption after drying. This might be due to the storage conditions (Chaiyasit *et al.*, 2007). The antioxidant treatment kept oxidation levels under the limit for up to 60 days. In Figure 4.11, the FFA (%) values for DPSH±AO stored for up to 60 days storage are represented.

The FFA values for DPSH exceeded the limit for acceptability after 14 days. However, antioxidant treatment kept FFA values below the acceptable limit for up to 30 days.

4.3.10 Microbiological Content

Mold colonies were not detected in the head samples stored for up to 60 days. This was most likely due to the water activity levels being below 0.6 in DPSH±AO. The TVC for DPSH±AO stored for up to 60 days are shown in Fig 4.12. The antioxidant treated samples had higher TVC due to higher TVC from wet fish heads. The TVC for DPSH±AO were below the limit determined to be acceptable for microbiological safety (Shewan, 1954; Tarr, 1954).

4.3.11 FAMEs

The FAMEs results for DPSH±AO are reported in Tables 4.7 and 4.8. The results pointed out that drying operation and ambient temperature storage without vacuum packaging potentially reduced polyunsaturated fatty acids in particular EPA and DHA in DPSH. However, the antioxidant treatment kept EPA and DHA levels higher in DPSH ($p < 0.05$) comparison to samples DPSH without antioxidant. The air exposure of fatty fish even at frozen storage was previously mentioned to oxidize readily fatty fish parts (Ke *et al.*, 1977).

High amounts of PUFAs together with the presence of heme pigments and trace amounts of metallic ions may lead to rancidity in salmon heads (Medina *et al.,* 2007), therefore packaging with oxygen barrier material would be included into the production system to prevent oxidation (Mol, 2005).

4.4 Conclusion

This study showed that an antioxidant treatment would be beneficial in delaying the oxidation of frozen and dried pink salmon heads. After some time, storage conditions that are freely permeable to air can cause the rapid oxidation of dried pink salmon heads even with antioxidant treatment. Salmon oils from both early run and late run pink salmon heads showed sufficiently high levels of EPA and DHA that could be refined for human consumption. Drying pink salmon heads, in combination with both antioxidant treatment and packaging that controls oxygen accessibility may retard the lipid oxidation for a longer period of time. Determination of the costs for antioxidant treatment would be necessary before development of commercially-dried products using pink salmon heads.

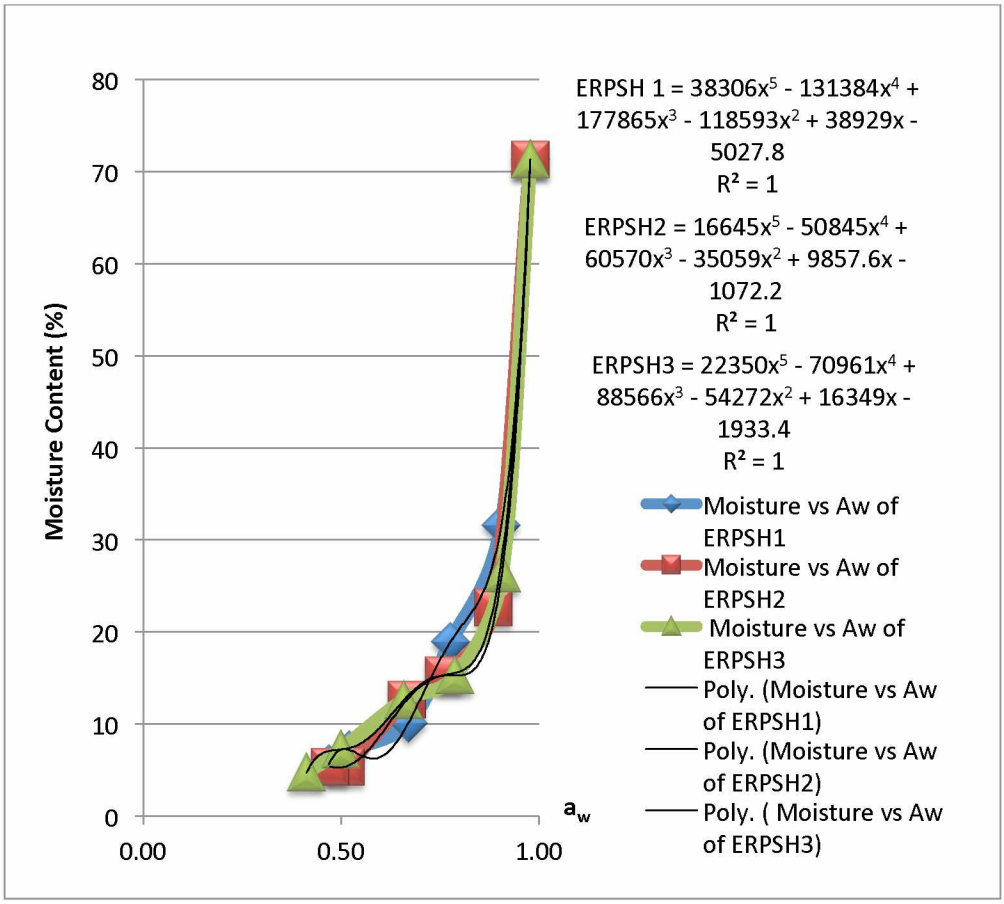

Figure 4.1 Moisture sorptions for early run pink salmon heads

ERPSH: Early Run Pink Salmon Heads.

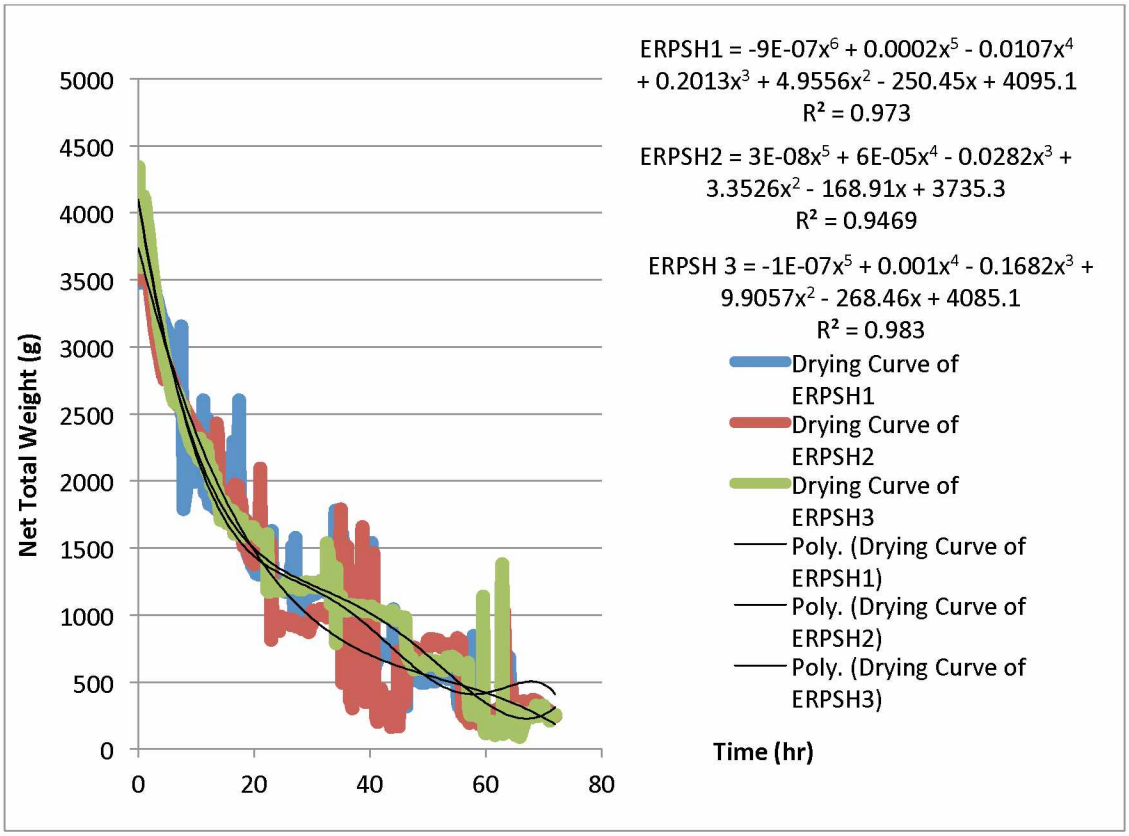

Figure 4.2 Drying curves for early run pink salmon heads

ERPSH: Early Run Pink Salmon Heads.

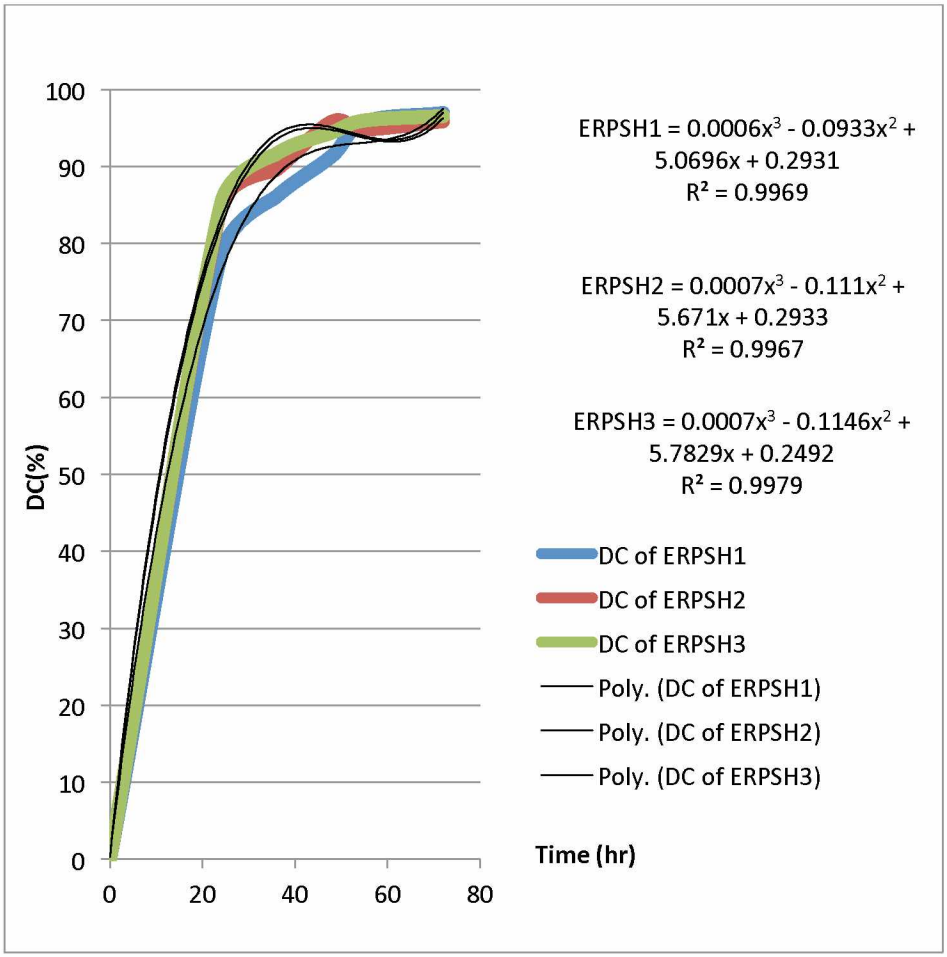

Figure 4.3 Drying coefficient (%) for early run pink salmon heads

ERPSH: Early Run Pink Salmon Heads; DC: Drying Coefficient.

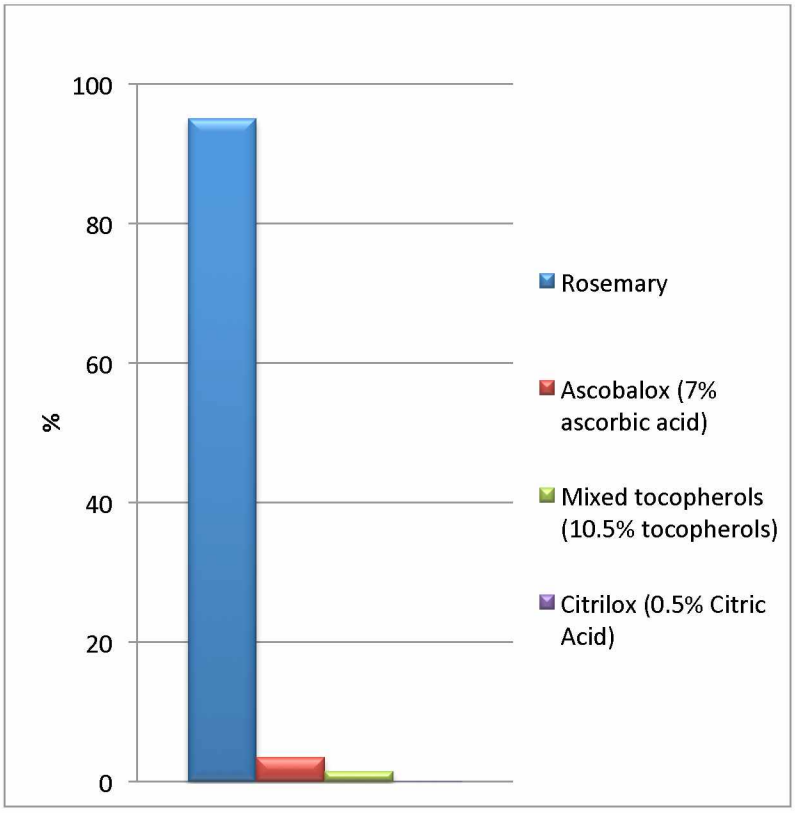

Figure 4.4 Inhibitors (%) of the antioxidant used for dipping solution

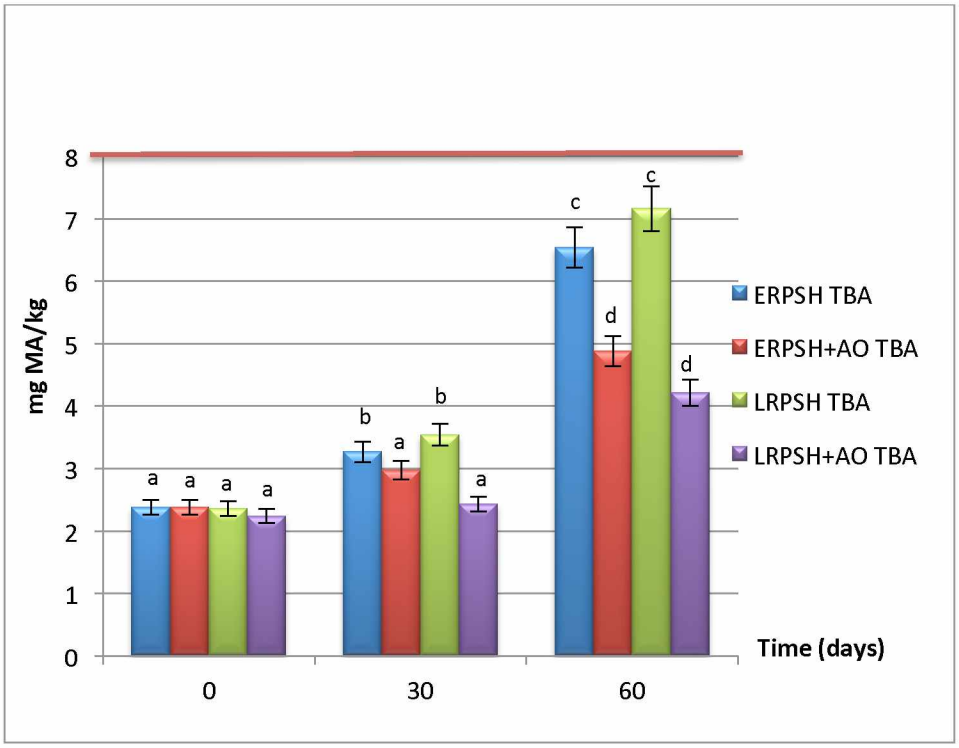

Figure 4.5 Thiobarbituric acid changes for early run pink salmon heads and late run pink salmon heads (+antioxidant) during frozen storage

n=6; ERPSH: Early Run Pink Salmon Heads; LPRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; Different letters represent for all data significant differences at $p < 0.05$. The red line depicts the maximum TBARS limit for human consumption.

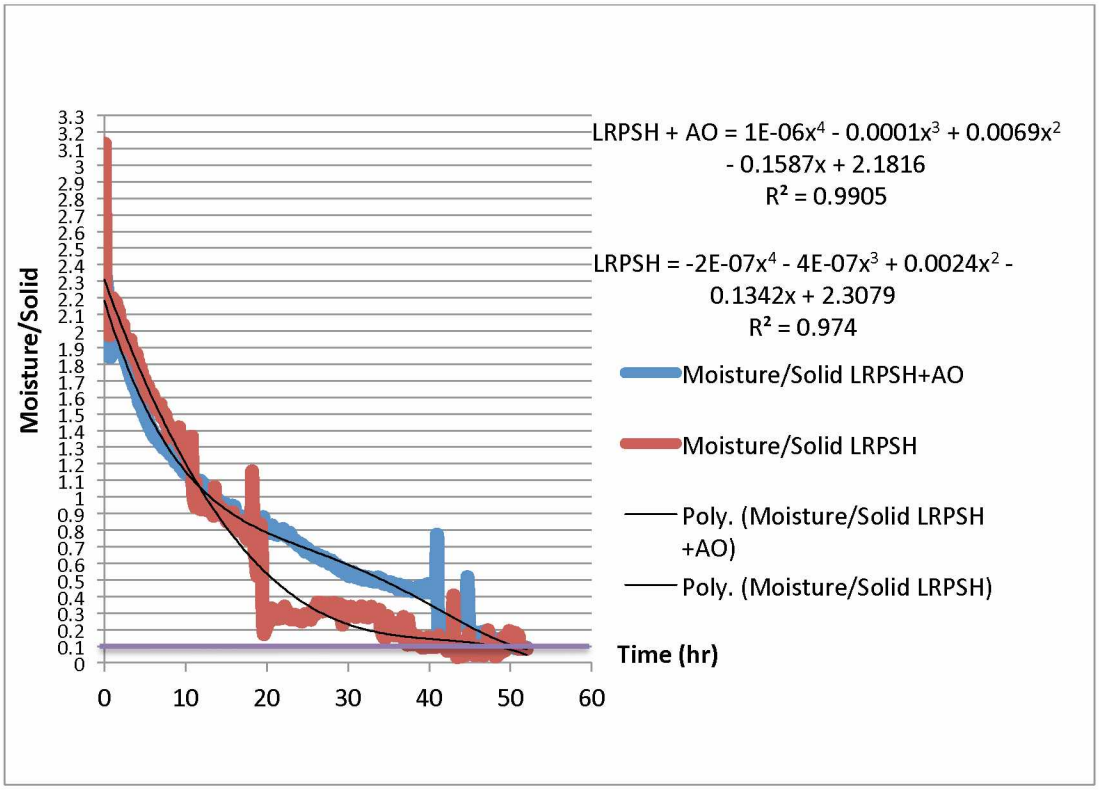

Figure 4.6 Moisture/solid ratio for late run pink salmon heads±antioxidant

LRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment. The purple line is the target moisture content.

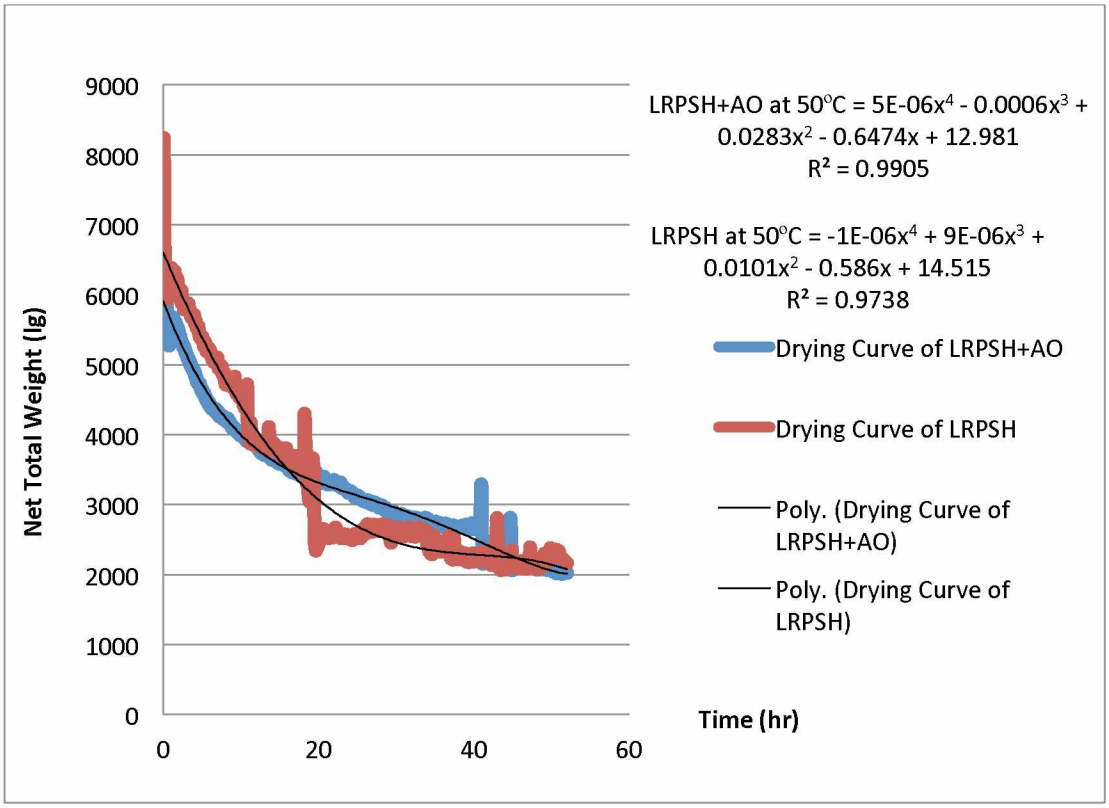

Figure 4.7 Drying curves for late run pink salmon heads±antioxidant

LRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment.

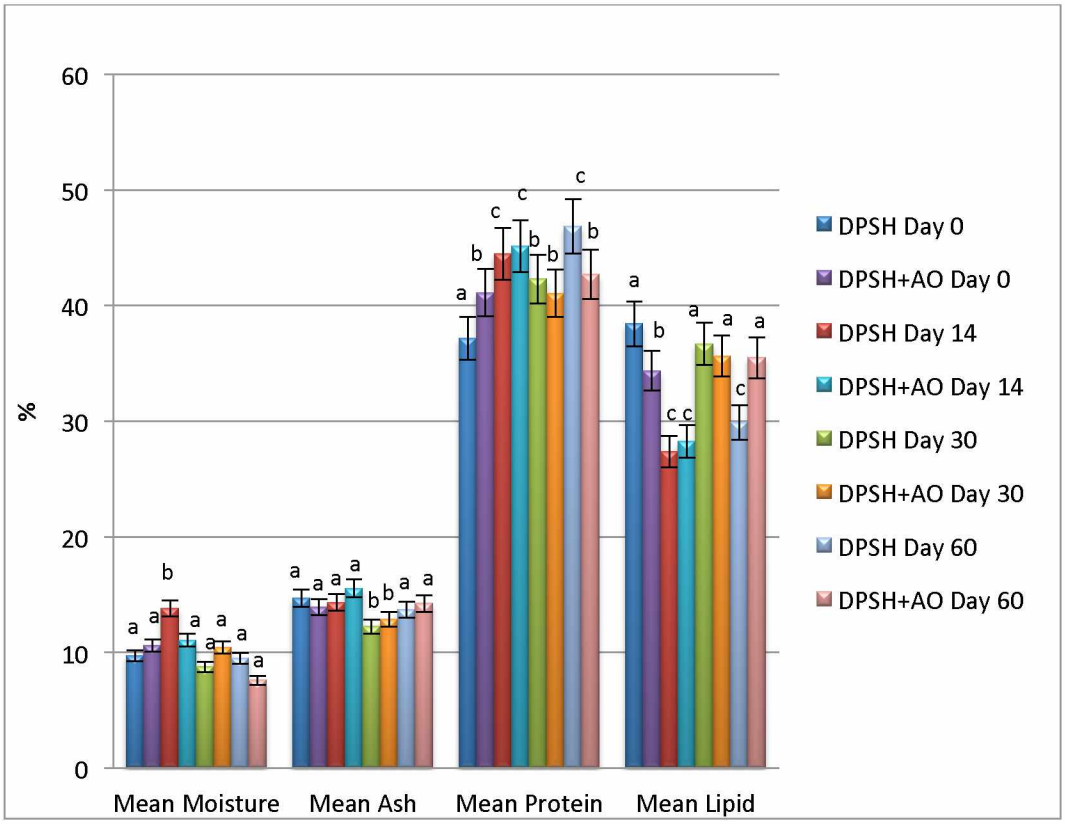

Figure 4.8 Proximate changes for dried pink salmon heads±antioxidant stored for up to 60 days

n= 6; DPSH: Late Run Dried Pink Salmon Heads; +AO: Antioxidant Treatment; Different letters represent significant differences at $p < 0.05$ for each category.

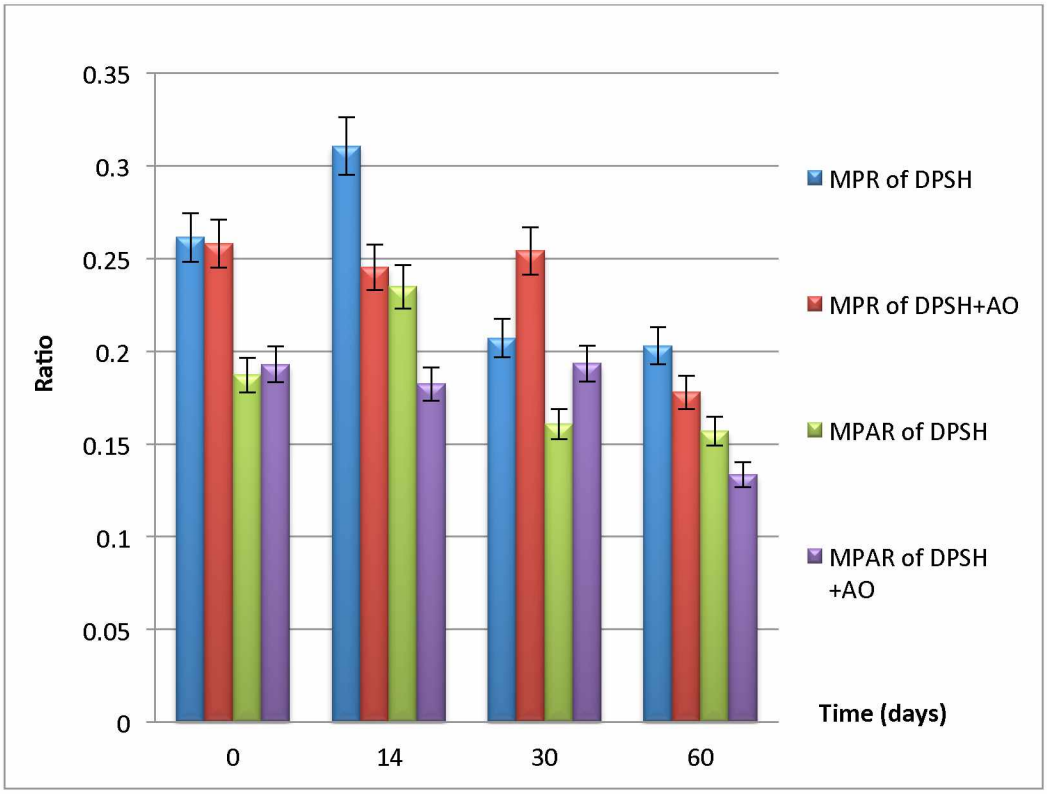

Figure 4.9 Moisture/protein ratio and moisture/protein+ash ratio for dried pink salmon heads±antioxidant stored for up to 60 days

n=6; DPSH: Late Run Dried Pink Salmon Heads; +AO: Antioxidant Treatment.

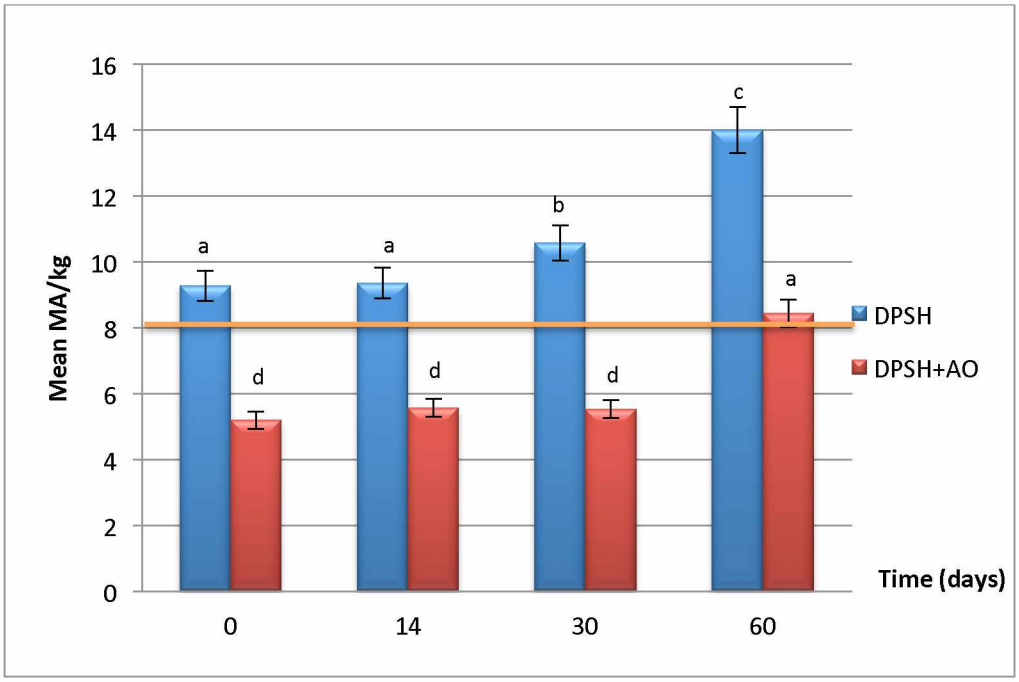

Figure 4.10 Thiobarbituric acid changes for dried pink salmon heads±antioxidant stored for up to 60 days

n=6; DPSH: Late Run Dried Pink Salmon Heads; +AO: Antioxidant Treatment; Different letters represent significant differences at $p < 0.05$. The orange line is the maximum TBARS limit for human consumption.

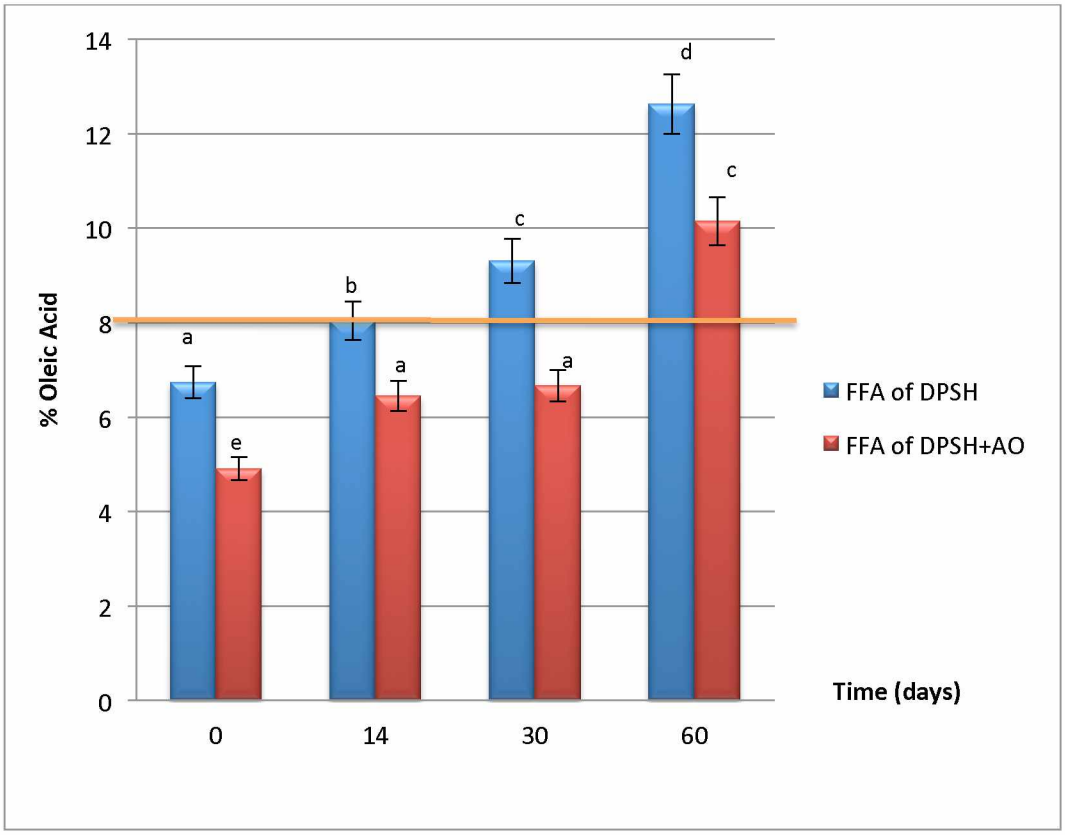

Figure 4.11 Free fatty acids (%) changes for dried pink salmon heads±antioxidant stored for up to 60 days

n=6; DPSH: Late Run Dried Pink Salmon Heads; +AO: Antioxidant Treatment; Different letters represent significant differences at $p < 0.05$. The orange line is the maximum FFA limit.

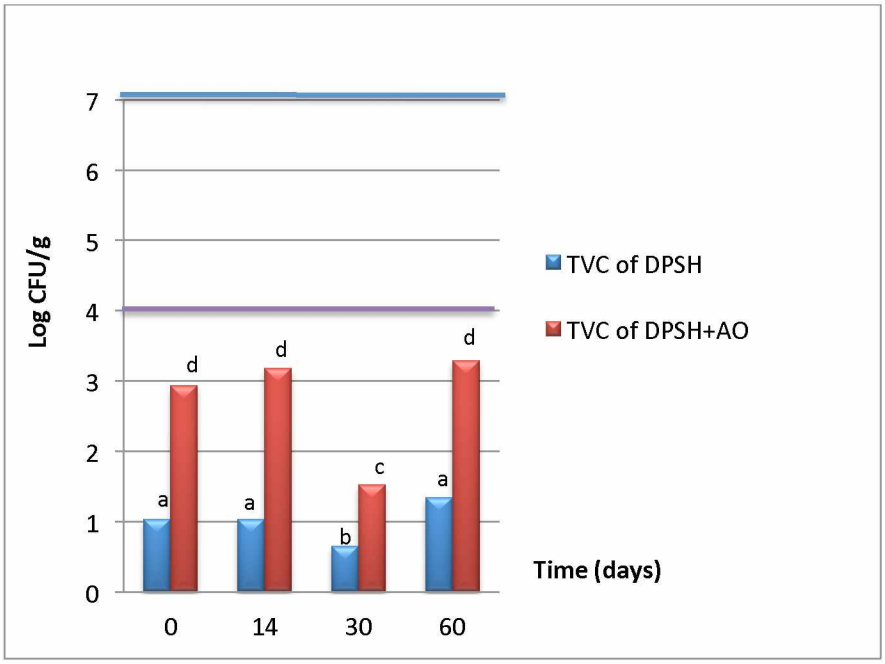

Figure 4.12 Changes in total viable counts for dried pink salmon heads±antioxidant stored for up to 60 days

n=6; DPSH: Late Run Dried Pink Salmon Heads; +AO: Antioxidant Treatment; Different letters represent significant differences at $p < 0.05$. The blue line is the limit for TVC (in frozen or fresh food) destined for human consumption. The purple line is the limit for TVC (in dried fish) destined for human consumption.

Table 4.1 Proximate analysis for early and late run pink salmon heads

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
ERPSH	1.0^a	0.0	71.4^a	1.1	9.4^a	0.9	14.0^a	0.8	5.2^a	0.2
LRPSH	1.0^a	0.0	73.8^b	2.4	6.3^b	1.3	15.8^b	1.3	4.1^b	0.8

n=6; ERPSH: Early Run Pink Salmon Heads; LRPSH: Late Run Pink Salmon Heads; SD: Standard Deviation; Different letters within column indicate statistical differences at $p < 0.05$.

Table 4.2 Proximate analysis for antioxidant treated early and late run pink salmon heads

Samples	a_w	SD	Moisture	SD	Lipid	SD	Protein	SD	Ash	SD
ERPSH+AO	1.0^a	0.0	71.7^a	0.8	10.6^a	0.9	12.6^a	1.4	5.1^a	0.2
LRPSH+AO	1.0^a	0.0	74.1^b	2.3	4.1^b	0.6	17.1^b	2.2	4.7^a	0.6

n=6; ERPSH: Early Run Pink Salmon Heads; LRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; SD: Standard Deviation; Different letters within column indicate statistical differences at $p < 0.05$.

Table 4.3 Moisture/protein ratio and moisture/protein+ash ratio for early and late run pink salmon heads±antioxidant treatment

Samples	Mean Moisture/Protein Ratio	SD	Mean Moisture/Protein+Ash Ratio	SD
ERPSH	5.1	0.3	3.7	0.2
LRPSH	4.8	0.5	3.8	0.5
ERPSH+AO	5.8	0.7	4.1	0.3
LRPSH+AO	4.4	0.7	3.4	0.5

n=6; ERPSH: Early Run Pink Salmon Heads; LRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; SD: Standard Deviation.

Table 4.4 Free fatty acids (%) for early and late run pink salmon heads±antioxidant treatment

Samples	Mean FFA (%)	SD
ERPSH	0.9	0.1
LRPSH	0.8	0.3
ERPSH+AO	0.6	0.1
LRPSH+AO	0.7	0.1

n=6; ERPSH: Early Run Pink Salmon Heads; LRPSH: Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; SD: Standard Deviation.

Table 4.5 Fatty acid methyl esters for early run pink salmon heads±antioxidant treatment

FAMEs	Mean Area (%) of ERPSH	SD	Mean Area (%) of ERPSH +AO	SD
14:0	4.5[a]	0.5	4.4[a]	0.7
16:0	18.9[a]	2.6	15.4[b]	1.8
16:1	3.6[a]	0.8	2.8[b]	0.4
18:0	3.0[a]	0.7	2.2[a]	0.5
18:1n9	13.1[a]	2.6	9.4[b]	1.3
18:2n6	1.1[a]	0.2	1.2[a]	0.3
20:5n3	11.8[a]	1.6	11.5[a]	0.9
22:6n3	14.5[a]	1.4	13.1[b]	1.0
24:1	0.8[a]	0.4	1.1[a]	0.3
ΣSAT	26.4[a]	1.2	22.0[b]	0.8
ΣMUFA	17.5[a]	1.7	13.3[a]	1.0
ΣPUFA	27.4[a]	0.7	25.8[a]	0.7
ΣP/S	1.0[a]	0.9	1.2[a]	0.7
Σω3	26.3[a]	0.8	24.6[a]	0.8
Σω6	1.1[a]	0.5	1.2[a]	0.6
Σω3/ ω6	24.0[a]	0.7	20.5[b]	0.7

n=6; ERPSH: Early Run Pink Salmon Heads; ERPSH +AO: Antioxidant Treated Early Run Pink Salmon Heads; SD: Standard Deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$.

Table 4.6 Fatty acid methyl esters for late run pink salmon heads±antioxidant treatment

FAMEs	Mean Area (%) of LRPSH	SD	Mean Area (%) of LRPSH +AO	SD
14:0	6.2[a]	0.4	5.6[a]	0.6
16:0	17.6[a]	1.9	16.3[b]	3.3
16:1	3.7[a]	0.6	3.6[a]	0.5
18:0	2.4[a]	0.3	2.4[a]	1.1
18:1n9	11.8[a]	2.7	10.5[b]	2.7
18:2n6	1.6[a]	0.2	1.8[a]	0.3
20:5n3	9.4[a]	1.3	10.8[b]	0.6
22:6n3	9.6[a]	1.3	11.7[b]	0.7
24:1	1.0[a]	0.4	0.9[a]	0.3
ΣSAT	26.2[a]	0.9	24.3[b]	1.5
ΣMUFA	16.5[a]	1.6	15.0[b]	1.8
ΣPUFA	20.6[a]	0.6	24.3[b]	0.4
ΣP/S	0.8[a]	0.7	1.0[a]	0.9
Σω3	19.0[a]	0.7	22.5[b]	0.4
Σω6	1.6[a]	0.3	1.8[a]	0.3
Σω3/ ω6	11.9[a]	0.5	12.5[a]	0.3

n=6; LRPSH: Late Run Pink Salmon Heads; LRPSH +AO: Antioxidant Treated Late Run Pink Salmon Heads; SD: Standard Deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$.

Table 4.7 Fatty acid methyl esters for dried pink salmon heads±antioxidant treatment

FAMEs	Mean Area (%) of DPSH Day 0	SD	Mean Area (%) of DPSH +AO Day 0	SD
14:0	5.3[a]	1.2	4.9[a]	0.3
16:0	15.1[a]	4.3	15.9[b]	1.4
16:1	3.3[a]	0.8	4.1[b]	0.8
18:0	0.7[a]	0.2	0.9[a]	0.3
18:1n9	13.3[a]	2.1	13.4[a]	1.6
18:2n6	2.9[a]	1.0	3.3[a]	0.3
20:5n3	7.2[a]	1.6	10.3[b]	2.1
22:6n3	7.7[a]	2.2	10.6[b]	0.6
ΣSAT	21.1[a]	6.4	21.7[a]	6.4
ΣMUFA	16.6[a]	6.0	17.5[a]	5.2
ΣPUFA	17.8[a]	3.1	24.2[b]	4.3
ΣP/S	0.8[a]	0.2	1.1[a]	0.4
Σω3	14.9[a]	3.5	20.9[b]	4.7
Σω6	2.9[a]	1.5	3.3[a]	1.7
Σω3/ ω6	5.1[a]	1.2	6.3[b]	1.4

n=6; DPSH: Dried Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; SD: Standard Deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$.

Table 4.8 Fatty acid methyl esters for dried pink salmon heads±antioxidant treatment stored for 30 days

FAMEs	Mean Area (%) of DPSH Day 30	SD	Mean Area (%) of DPSH+AO Day 30	SD
14:0	5.9[a]	0.7	4.8[b]	0.3
16:0	16.2[a]	2.5	17.7[b]	2.8
16:1	4.1[a]	0.5	4.4[a]	0.4
18:0	1.3[a]	0.5	1.3[a]	0.2
18:1n9	11.4[a]	1.1	13.9[b]	2.3
18:2n6	1.7[a]	0.1	3.4[b]	0.8
20:5n3	4.4[a]	0.4	6.9[b]	0.5
22:6n3	5.5[a]	0.5	8.1[b]	1.1
ΣSAT	23.4[a]	6.6	23.8[a]	7.3
ΣMUFA	15.5[a]	2.0	18.3[b]	5.1
ΣPUFA	11.6[a]	0.9	18.4[b]	3.1
ΣP/S	0.5[a]	0.2	0.8[a]	0.2
Σω3	9.9[a]	2.1	15.0[b]	3.2
Σω6	1.7[a]	1.5	3.4[a]	1.3
Σω3/ ω6	5.8[a]	0.7	4.4[b]	1.3

n=6; DPSH: Dried Late Run Pink Salmon Heads; +AO: Antioxidant Treatment; SD: Standard Deviation; ΣSAT: Total Saturated Fatty Acids; ΣMUFA: Total Monounsaturated Fatty Acids; ΣPUFA: Total Polyunsaturated Fatty Acids; ΣP/S: Total Polyunsaturated Fatty Acids/Saturated Fatty Acids; Different letters in a row represent significant differences at $p < 0.05$.

4.5 References

AOAC, 2005. Official methods of analysis of AOAC International. 18th edition. Association of Official Analytical Chemists International, Arlington. VA.

AOCS, 1998. Official methods and recommended practices of the American Oil Chemists' Society (5th Edition). Champaign, Illinois, Washington, DC.

Bimbo P.A., 2009. Alaska seafood byproducts: Potential products, markets and competing products. Report for Alaska Fisheries Development Foundation. Anchorage, AK. pp. 227.

Chaiyasit W., Elias R.J., McClements D.J. and Decker E.A., 2007. Role of physical structures in bulk oils on lipid oxidation. Crit. Rev. Food Sci. Nut. 47: 299-317.

Doe P.E., 1998. Fish drying and smoking: Production and quality. Technomic Publishing Inc. UK. pp. 250.

Frankel E.N., 1993. Viewpoint: In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends Food Sci. Tech. 4: 220-224.

Frankel E.N. and Huang S.W., 1996. Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. J. Sci. Food Agr. 72: 201-208.

Freeman D.W. and Hearnsberger D.O., 1994. Rancidity in selected sites of frozen catfish fillets. J. Food Sci. 69(1): 60-63.

ICMSF, 1978. Microorganisms in foods. Vol. 1. The International Commission on Microbiological Specifications for Foods. Toronto, ON, Canada. University of Toronto Press. pp. 343.

Ke P.J., Ackman R.G., Linke B.A. and Nash D.M., 1977. Differential lipid oxidation in various parts of frozen mackerel. J. Food Tech. 12: 37-47.

Lemon D.W., 1975. An improved TBA test for rancidity. Environment Canada. Fisheries and Marine Service. New Series Circular No. 51: 52-55. Halifax, Nova Scotia.

Lopez J.F., Zhi N., Carbonell L.A., Alvarez J.A.P. and Kuri V., 2005. Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. Meat Sci. 69: 371-380.

Marriott N.G. and Gravani R.B., 2006. Sanitation and the food industry. Chapter 1. In: Principles of food sanitation. 5th edition. Springer. UK. pp. 15.

Maxwell J.R. and Marmer N.W., 1983. Methods: Systematic protocol for the accumulation of fatty acid data from multiple tissue samples: Tissue handling, lipid extraction and class separation, and capillary gas chromatographic analysis. Lipids. 18(7): 453-459.

McClements D.J. and Decker E.A., 2000. Lipid oxidation in oil-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food system. J. Food Sc. 65(8): 1270-1282.

Medina I., Gallardo J.M., Gonzales M.J., Lois S. and Hedges N., 2007. Effect of molecular structure of phenolic families as hydroxycinnamic acids and catechins on their antioxidant effectiveness in minced fish muscle. J. Agr. Food Chem. 55: 3888-3895.

Messer J.W., Peeler J.T. and Gilchrist J.E., 1984. Aerobic plate count. Chapter 4. In: Bacteriological Analytical Manual. Food and Drug Administration. Association of Official Analytical Chemists. Arlington, VA.

Mol S., 2005. Preparation and the shelf life assessment of ready-to-eat fish soup. Eur. Food Res. Tech. 220: 305-308.

Reid R.A., Durance T.D., Walker D.C. and Reid P.E., 1993. Structural and chemical changes in the muscle of chum salmon during spawning migration. Food Res. Int. 26: 1-9.

Sathivel S., Liu Q., Huang J. and Prinyawiwatkul W., 2007. The influence of chitosan glazing on the quality of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. J. Food Eng. 83: 366-373.

Schormuller J., 1968. Handbuch der lebensmittelchemie (Band III/2). Springer Verlag. Berlin Heidelberg. New York. Handbook of Food Chem. pp. 1341-1397.

Serdaroglu M. and Felekoglu E., 2005. Effects of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. J. Food Quality. 28: 109-120.

Shahidi F. and Botta J.R., 1994. Seafoods: Chemistry, processing, technology and quality. Chapman and Hall. London, UK. pp. 342.

Shewan J.M., 1954. The bacteriology of dehydrated fish. III. Observations and experiments made during small scale commercial production. J Hyg. 52(2): 247-252.

Tarr H.L.A., 1954. Microbiological deterioration of fish post mortem, its detection and control. Microbiol. Mol. Biol. Rev. 18(1): 1-15.

Vareltzis K., Koufidis D., Gavriilidou E., Papavergou E. and Vasiliadou S., 1997. Effectiveness of a natural rosemary (*Rosmarinus officinalis*) extract on the stability of filleted and minced fish during frozen storage. Z. Lebensm. Unters. Forsch A. 205: 93-96.

Wada S. and Fang X., 1992. The synergistic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. J. Food Proc. Pres. 16: 263-274.

Chapter 5. General Conclusions

Overall, these results suggest that both pink salmon and Pacific cod heads display biochemical differences that can influence the options for their utilization. Both pink salmon and Pacific cod heads are abundant in Kodiak, AK. Pacific cod heads are larger, heavier, and leaner, affecting their shelf stability as either a frozen and or dried product when compared to frozen and dried pink salmon heads. Pink salmon and red salmon heads contained higher amount of polyunsaturated fatty acids (PUFA). Antioxidant dipping (2% v/v) can be effective in extending shelf life of pink salmon heads frozen and maintained at -40°C and dried pink salmon heads held at ambient temperature storage for not longer than 60 days. Air-drying appears to be an accelerating factor in lipid oxidation for pink salmon heads. The PUFA levels in dried pink salmon significantly decreased throughout ambient storage compared to dried Pacific cod heads. These findings suggested that extracting lipids from frozen pink salmon heads treated with antioxidant would be more effective option for processors. Frozen Pacific cod heads are already being shipped to Asian markets from Kodiak, AK. However, shipping dried Pacific cod heads might be a cost effective option through the reduction of the costs of refrigeration. Salmon oils can be extracted from salmon heads and used for human consumption as a healthful food additive or as a nutraceutical via encapsulation. Chondroitin sulfate can be extracted from the cartilage of salmon heads. Therefore, dried pink salmon heads, treated with an antioxidant dipping could potentially be exported profitably to East African markets to be used in fish head soup.

Appendix A. Experimental Design of Drying Pink and Red Salmon Heads

Sampling of Pink and Red Salmon Heads

- Collect fresh Pacific pink salmon heads (*Oncorhynchus gorbuscha*) and red salmon (*Oncorhynchus nerka*) heads from local processor.
- Deliver them immediately to FITC Pilot Plant.
- Remove the gills.
- Prepare box with plastic sheeting.
- Layer the heads in box.
- Label the box and store it in the freezer at -40°C (Blast freezer).

Analyses of Wet Pink and Red Salmon Heads

- Thaw 6 wet frozen pink and red salmon heads (six replicates).
- Split the heads into halves.
- Cut each individual heads into 2 cm pieces.
- Grind for each head using Cuisine-Art Grinder.
- Pack them into the ZipLock bags and label each packages in six replicates.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV, and FFA).
- Run microbiological analyses of wet samples.

Minimum Required Material (g) for Wet Pink and Red Salmon Heads

✓Water activity (a_w), Moisture, Ash Content and Protein Content	= 7
✓Lipid Extraction (ASE), and Free Fatty Acid (FFA)	= 5
✓Peroxide Value (PV) and Thiobarbituric Acid (TBA) Assays	= 5.5
✓Microbiological Analysis (TVC and Mold Growth)	= 5
✓Total Required Material from Each Wet Head Weight	= 67.5

Drying Pink and Red Salmon Heads for Acquiring Drying Curve

- Thaw 30 frozen pink and red salmon heads at cooler.
- Split the heads into halves.
- Record the weight of each rack with heads.
- Set the drying time and temperature and software.
- Record the initial total weight.
- Start drying.
- Measure the air velocity of the drying machine.
- Remove the samples each timeline to determine the weight, moisture content and a_w.
- Plot the drying curve into the computer.
- Record the final weight of each rack.

Note: Red salmon heads were dried only at 50°C up to 72 hours.

Drying Pink and Red Salmon Heads

- Thaw 50 pink and red salmon heads.
- Split them into the halves.

- Record the weight of each rack with heads. (Put 10 halves on each rack).
- Record the initial total weight.
- Start drying.
- Calculate the target weight based on the target moisture content (10 %) and a_w (< 0.6).
- Plot the drying curve into the computer.
- Record the final weight of each rack.
- Pack them into the double gusset bags and stored them at ambient temperature at shed (open to the air).

Analyses of Dried Pink Salmon Heads

- Remove 6 dried pink salmon and red salmon heads from the incubator for analyses at each shelf life time-point.
- Cut each individual heads into 2 cm pieces.
- Grind 2 cm pieces for each dried head samples.
- Pack them into the ZipLock bags and label each packages.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV and FFA).
- Run microbiological analyses (TVC and mold colonies count) of dried samples.

Minimum Required Material (g) for Dried Pink and Red Salmon Heads

- ✓ Water activity (a_w), Moisture, Ash Content, Protein Content = 7
- ✓ Lipid Extraction (ASE), and Free Fatty Acid (FFA) = 5

- ✓ Peroxide Value (PV) and Thiobarbituric Acid (TBA) Assays = 5.5
- ✓ Microbiological Analysis (TVC and Mold Growth) = 2
- ✓ Total Required Material for Each Dried Head Weight = 19.5

- Note: All biochemical and microbiological analyses will be performed according to the Materials and Methods.

Appendix B. Experimental Design of Drying Pacific Cod Heads

Sampling of Pacific Cod Heads

- Collect fresh Pacific cod heads (*Gadus macrocephalus*) heads from local processor.
- Deliver them to FITC Pilot Plant.
- Remove the gills.
- Prepare box with plastic sheeting.
- Layer the heads in box.
- Label the box and store it in the freezer at -40°C (Blast freezer).

Analysis Procedure of Wet Pacific Cod Heads

- Thaw 6 wet frozen Pacific cod heads (six replicates).
- Split the heads into halves.
- Cut each individual heads into 2 cm pieces.
- Grind 2 cm pieces for each head using Cuisine-Art Grinder
- Pack them into the ZipLock bags and label each packages in six replicates.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV, FFA and FAME).
- Run microbiological analyses of wet samples.

Minimum Required Material (g) for Wet Pacific Cod Heads

✓ Water activity (a_w), Moisture, Ash Content and Protein Content = 7

✓ Lipid Extraction (ASE), FAME and Free Fatty Acid (FFA) = 5

- ✓ Peroxide Value (PV) and Thiobarbituric Acid (TBA) Assays = 5.5
- ✓ Microbiological Analysis (TVC and Mold Growth) = 5
- ✓ Total Required Material from Each Wet Pacific Cod Head Weight = 67.5

Drying Pacific Cod Heads for Acquiring Drying Curve

- Thaw 36 frozen Pacific cod heads at cooler.
- Split the heads into halves.
- Record the weight of each rack with heads.
- Record the initial total weight.
- Start drying.
- Measure the air velocity of the drying machine.
- Remove the samples at the end to determine the weight, moisture content and a_w.
- Plot the drying curve into the computer.
- Record the final weight of each rack.

Drying Pacific Cod Heads

- Thaw 50 Pacific cod heads.
- Split them into halves.
- Record the weight of each rack with heads. (Put 10 halves on each rack).
- Record the initial total weight.
- Start drying at 50&30°C combination.
- Calculate the target weight based on the target moisture content (10-15%) and a_w (< 0.6).
- Plot the drying curve into the computer.

- Record the final weight of each rack.
- Pack them into the double gusset bags and stored them at ambient temperature in the incubator.

Analyses of Dried Pacific Cod Heads

- Remove 6 dried Pacific cod heads from the incubator for analyses at each shelf life time-point.
- Cut each individual heads into 2 cm pieces.
- Grind for each dried head samples using Wiley mill.
- Pack them into the ZipLock bags and label each packages.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV and FFA).
- Conduct FAME analysis at day 0, 90 and 180.
- Run microbiological analyses (TVC and mold colonies count) of dried samples.

Minimum Required Material (g) for Dried Pacific Cod Heads

✓	Water activity (a_w), Moisture, Ash Content, Protein Content	= 7
✓	Lipid Extraction (ASE), FAME and Free Fatty Acid (FFA)	= 5
✓	Peroxide Value (PV) and Thiobarbituric Acid (TBA) Assays	= 5.5
✓	Microbiological Analysis (TVC and Mold Growth)	= 2
✓	Total Required Material for Each Dried Pacific Cod Head Weight	= 19.5

- Note: All biochemical and microbiological analyses will be performed according to the Materials and Methods. Experimental for pink salmon heads is similar to Appendix A and C.

Appendix C. Experimental Design of Drying Pink Salmon Heads

Sampling of Pink Salmon Heads

- Collect fresh pink salmon (*Onchorynchus gorbuscha*) heads from local processor.
- Deliver them to FITC Pilot Plant.
- Remove the gills.
- Prepare box with plastic sheeting.
- Layer the heads in box.
- Label the box and store it in the freezer at -40°C (Blast freezer).

Analysis of Wet Pink Salmon Heads (EARLY RUN)

- Thaw 6 wet frozen early run pink salmon heads.
- Split the heads into halves.
- Cut each individual heads into 2 cm pieces.
- Grind 2 cm pieces for each head using Cuisine-Art Grinder
- Pack them into the ZipLock bags and label each packages in six replicates.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV and FFA).
- Run microbiological analyses of wet samples.

Minimum Required Material (g) for Wet Pink Salmon Heads

✓Water activity (a_w), Moisture, Ash Content and Protein Content = 7

✓Lipid Extraction (ASE) and Free Fatty Acid (FFA) = 5

✓Peroxide Value (PV) and Thiobarbituric Acid (TBA) Assays = 5.5

✓Microbiological Analysis (TVC and Mold Growth) = 5

✓ Total Required Material from Each Wet Head Weight = 67.5

Drying Pink Salmon Heads (Early Run) for Acquiring Drying Curve

- Thaw 36 frozen heads at cooler.
- Split the heads into halves.
- Record the weight of each rack with heads.
- Set the drying time and temperature and software up to 72 hours.
- Record the initial total weight.
- Start drying.
- Measure the air velocity of the drying machine.
- Remove the samples each timeline to determine the weight, moisture content and a_w.
- Plot the drying curve into the computer.
- Record the final weight of each rack.

Analyses of Wet Pink Salmon Heads

- Collect fresh late run pink salmon (*Onchorynchus gorbuscha*) heads from local processor.
- Deliver them to FITC Pilot Plant.
- Remove the gills.
- Prepare box with plastic sheeting.
- Layer the heads in box.
- Label the box and store it in the freezer at -40°C (Blast freezer).

- Thaw out 6 wet head samples.
- Split the heads into halves.
- Cut each individual heads into 2 cm pieces.
- Grind 2 cm pieces for each wet head samples.
- Pack them into the ZipLock bags and label each package.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA, PV and FFA).
- Run microbiological analyses of wet samples.

Drying Pink Salmon Heads (Control Group)

- Thaw 50 pink salmon heads.
- Split them into halves.
- Record the weight of each rack with heads. (Put 10 halves on each rack).
- Record the initial total weight.
- Start drying.
- Calculate the target weight based on the target moisture content (10-15%) and a_w (< 0.6).
- Plot the drying curve into the computer.
- Record the final weight of each rack.
- Pack them into the double gusset bags and stored them at ambient temperature at shed (open to the air).
- Mimicking the African style of storage.

Drying Pink Salmon Heads (Antioxidant Treatment)

- Thaw 50 late run pink salmon heads.
- Split them into halves.
- Run a preliminary test on Duralox natural herb antioxidant blend (MAN-5) to determine the uptake and the concentration in the chilled distilled water to estimate the % Duralox in the dipping solution.
- Prepare 2% v/v of antioxidant solution in distilled hot water.
- Record the initial weight of each heads.
- Immerse those head samples into the antioxidant solution for two minutes.
- Filter them and make sure there is no dripping.
- Record the end weight of each heads.
- If necessary prepare another 2% v/v of antioxidant solution in distilled water.
- Calculate the antioxidant uptake.
- Record the weight of each rack with heads. (Put 10 halves on each rack).
- Record the initial total weight.
- Start drying.
- Calculate the target weight based on the target moisture content (10-15%) and a_w (< 0.6).
- Plot the drying curve into the computer.
- Record the final weight of each rack.

- Pack them into the double gusset bags and tie the bags and store them at the ambient temperature allowing air to circulate among dried heads until conducting the analyses.

Analyses of Dried pink salmon heads

- Remove 6 dried pink salmon heads (control) and 6 dried pink salmon heads (anti-oxidant added) for analyses at each shelf life time-point.
- Cut each individual heads into 2 cm pieces.
- Grind 2 cm pieces for each dried head samples.
- Pack them into the ZipLock bags and label each packages.
- Conduct the proximate composition of wet samples.
- Conduct oxidation analyses (TBA and FFA).
- Conduct FAME analysis at day 0 and 30 days.
- Run microbiological analyses (TVC and mold colonies count) of dried samples.

Minimum Required Material (g) for Dried Pink Salmon Heads

✓	Water activity (a_w), Moisture, Ash Content, Protein Content	= 7
✓	Lipid Extraction (ASE), Free Fatty Acid (FFA)	= 5
✓	Thiobarbituric Acid (TBA) Assays	= 5
✓	Microbiological Analysis (TVC and Mold Growth)	= 2
✓	Total Required Material for Each Dried Head Weight	= 19

- Note: All biochemical and microbiological analyses will be performed according to the Materials and Methods.